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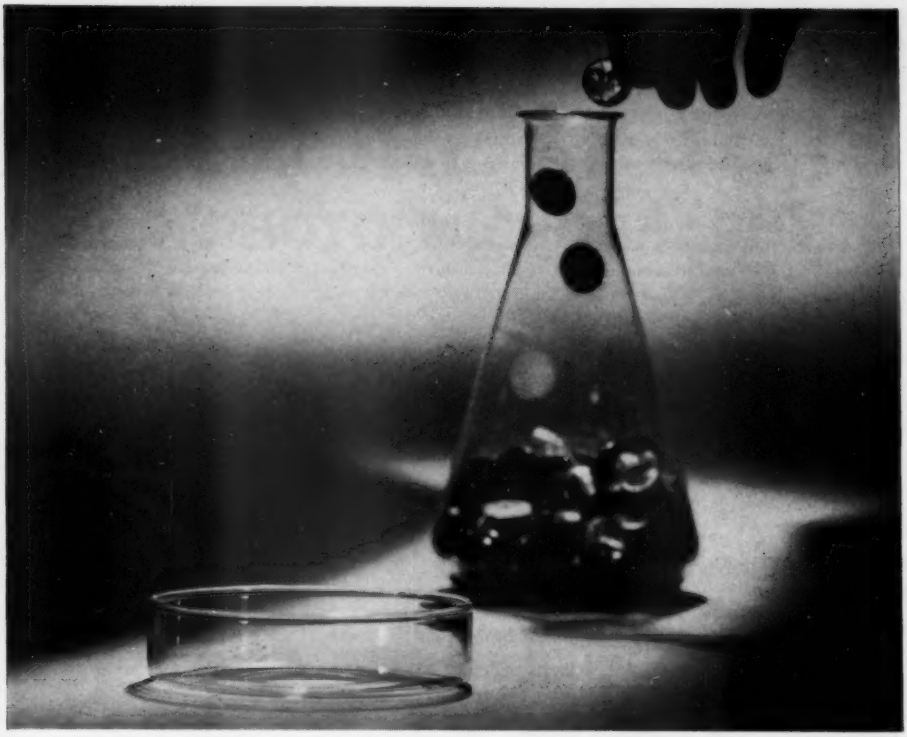

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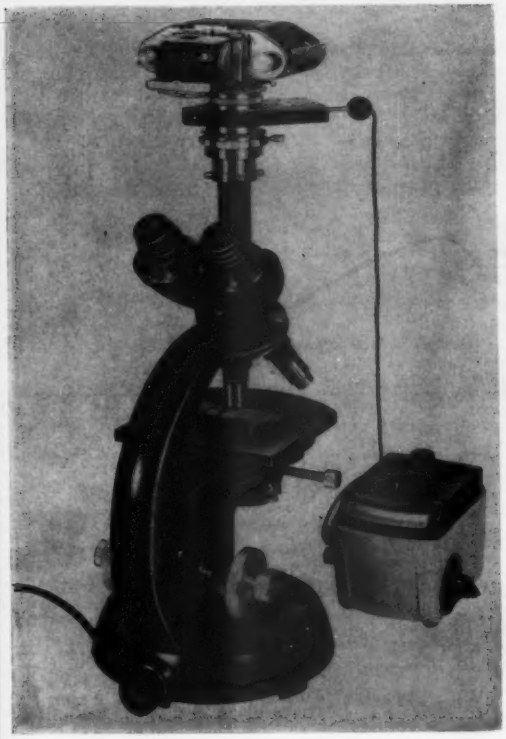

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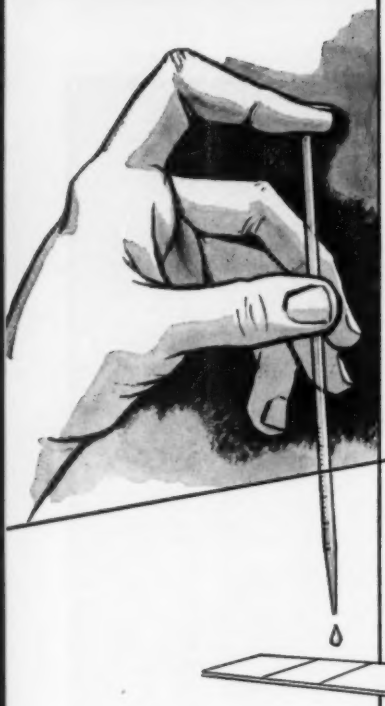

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A.M.A. ARCHIVES OF
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Glomerular Capillary Endotheliosis in Toxemia of Pregnancy

BENJAMIN SPARGO, M.D.; CHARLES P. McCARTNEY, M.D., and ROBERT WINEMILLER, A.B., Chicago

While the identification of individual cases of toxemia of pregnancy on the basis of the clinical findings is currently accepted, the exclusion of those cases in their last trimester of pregnancy with edema, proteinuria, and hypertension having their origin in a preexisting renal vascular disease is not always possible.[1] The potentially serious nature of the sequelae of these latter states serves to emphasize the desirability of a more satisfactory separation of these conditions.

Pathologic evidence which would clarify this situation with an acceptable single renal lesion has not been agreed upon. The transient nature of the glomerular lesion which has been most consistently associated with preeclampsia-eclampsia, with its early reversibility following delivery, has limited the use of autopsy material to an occasional fatal case in which death occurs while symptoms are present. The general inaccessibility of the kidney has made adequate serial sampling impossible until the recent use of the percutaneous needle biopsy. In sizable series using needle biopsy material, light microscopy has not permitted resolution of this problem, and special staining methods by a variety of workers have failed to resolve the disagreement as to the degree of involvement of each component of the glomerulus.[2,3]

The recent development of techniques for making ultrathin tissue sections, and the development of reliable commercial equipment have led to the clinical use of the electron microscope for the study of renal biopsy tissues. This makes possible some clarification of the principal site and type of the glomerular pathologic change which has been debated for the past 50 years. It is hoped that the use of this new tool, with the study of serial renal biopsy specimens, will permit more specific diagnoses and contribute to an understanding of the pathogenesis of the glomerular lesions associated with the various clinical entities which comprise the toxemias of pregnancy.

Material and Methods

Fourteen patients in late pregnancy had biopsies. Of the group, one had another biopsy 11 days, and again 4 weeks, after delivery. These were part of an electron microscopic renal biopsy series in progress for two years. The material for electron microscopy was fixed in buffered osmic acid,[4] dehydrated in alcohols, and embedded in butyl methacrylate, and then polymerized by ultraviolet light. Ultrathin sections were cut, using a plate glass knife on a Servel Porter-Blum microtome,[5] and viewed on an RCA (Model EMU-3) electron microscope at magnifications of from 4,000 to 20,000. Material for light microscopy was fixed in Helly's fluid, embedded in paraffin, and the sections stained with hematoxylin and eosin, the

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From the Department of Pathology and the Department of Obstetrics and Gynecology, The University of Chicago School of Medicine.

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periodic acid-Schiff technique (PAS), and Mallory's trichrome stain.

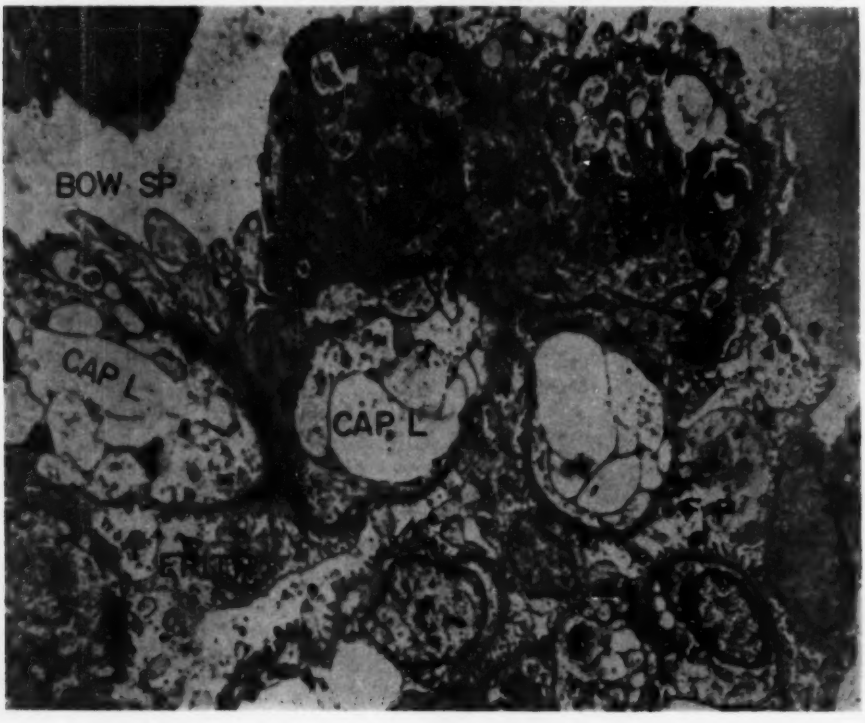
Results

A consistent lesion was found by electron microscopy in the glomeruli of five women (Table) having a clinical diagnosis of preeclampsia-eclampsia. This glomerular lesion was diffuse and uniform; and, although it varied in severity from case to case, it was easily discernible and different from the change found in glomerulonephritis. In these instances in which the toxemic lesion was present, light microscopy revealed swollen, ischemic glomeruli with thickened capillary walls. The glomerular components involved in this capillary thickening could not be accurately identified by this means. As a consequence, this lesion could only be classified in the general category of membranous glomerulonephritis, and the resolution afforded by the electron microscope was required to determine its exact morphology. With electron microscopy

Electron Microscopic Findings in Pregnant Women

	Age, Yr.	Gravida	Para	Clinical Diagnosis	Electron Microscopy
1	19	1	1	Preeclampsia	Glomerular endotheliosis
2	19	1	1	Preeclampsia	Glomerular endotheliosis
3	30	1	1	Preeclampsia	Glomerular endotheliosis
4	34	3	3	Preeclampsia	Glomerular endotheliosis
5	36	10	7	Hypertensive disease; twins; abruptio placentae	Nephrosclerosis
6	32	4	3	Preeclampsia	Glomerular endotheliosis
7	23	3	1	Hypertensive disease; abruptio placentae; afibrinogenia	Tubulorhexic lesions; nephrosclerosis
8	38	11	8	Hypertensive disease	Nephrosclerosis
9	22	6	6	Chronic renal disease	Chronic glomerulonephritis
10	23	3	2	Excess weight gain	Normal
11	40	4	4	Normal pregnancy	Normal
12	40	5	5	Normal pregnancy	Normal
13	41	5	4	Normal pregnancy	Normal
14	37	4	4	Normal pregnancy; previous eclampsia	Normal

Fig. 1.—A typical ischemic glomerular lobule from a case of preeclampsia. There is marked narrowing of the capillary lumen by the cytoplasm of the endothelial cell. It is clear that the basement membrane is intact and the epithelial cell change is slight. × 4,500.



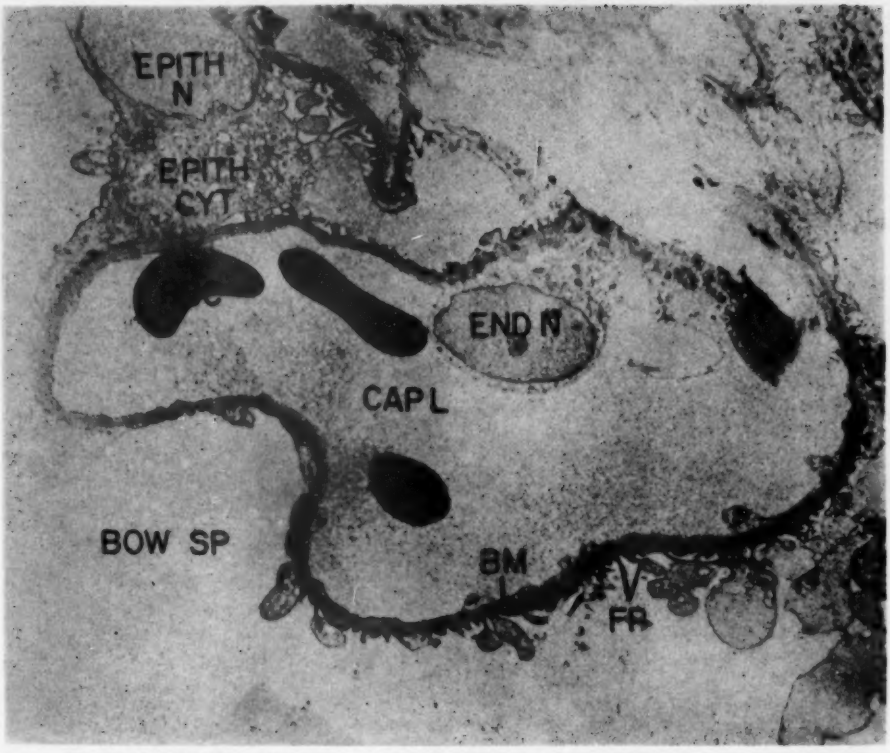


Fig. 2.—Cross section of a normal glomerular capillary, showing the widely patent capillary lumen and scanty endothelial cell cytoplasm. × 5,000.

the capillary lumens were very much narrowed, owing to an increase in endothelial cytoplasm (Fig. 1). The cytoplasm had a variety of changes, including vacuolation, droplet formation, cytoplasmic strands, membrane condensation, and an increase in particulate structures. The cytoplasmic strands, although approaching the basement membrane, could clearly be seen to be separate. The endothelial nuclei showed mild proliferative changes. The capillary loops most severely involved were dilated, but the basement membrane was consistently normal. The epithelial cells had no marked foot-process change. The cytoplasm contained a few scattered vacuoles but did not seem primarily involved in the process, and it was without reticulation. The arterioles and small arteries showed no consistent change. The tubular lumens contained casts; the tubular cells in the proximal portion frequently were swollen, and the cytoplasm contained hyaline droplets.

In the one case in which a second biopsy was done 11 days after delivery the endothelial cells of the glomerular capillaries had cleared in areas, but many capillary loops contained coalescent vacuoles and a faintly stained network partially occluding the lumen(Fig. 5). Casts were not conspicuous, and tubular cells appeared normal. A biopsy four weeks following delivery showed normal capillary lumens, and the endothelial cytoplasm was not prominent (Fig. 6).

This lesion contrasts with that of a case of chronic glomerulonephritis of this series. In the latter, the endothelial-cell proliferation was marked, and there was considerable thickening and folding of the basement

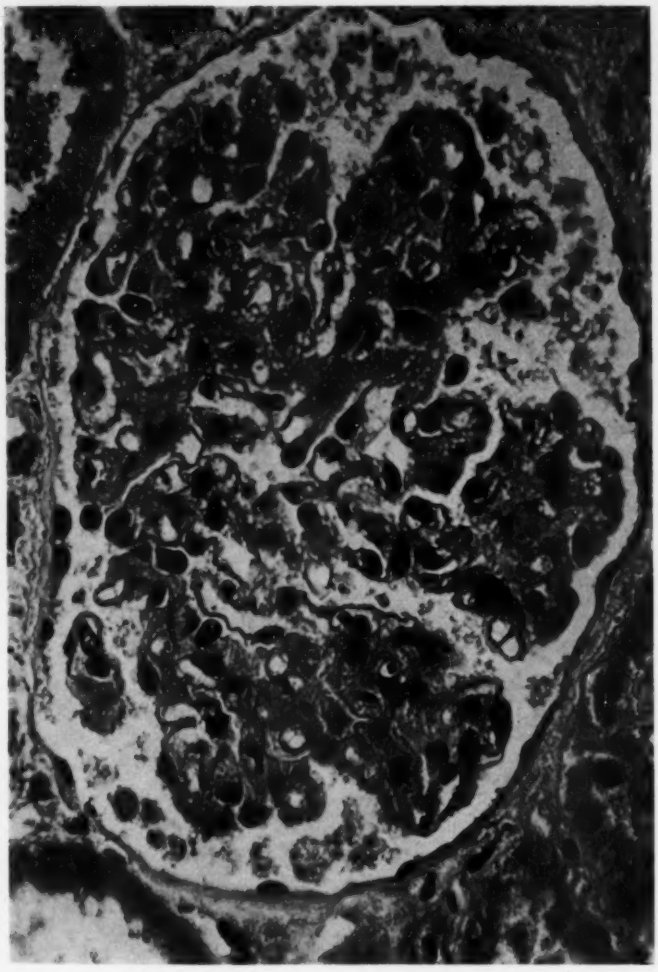


Fig. 3.—A typical glomerulus from a patient with preeclampsia at term. Light microscopy shows the glomerular abnormality, with thickening of glomerular capillaries. Hematoxylin-eosin stain; ×850.

membrane in the so-called mesangial areas, with fusion of glomerular capillaries in many lobules. Epithelial-cell proliferation in this case was mild, with no crescent formation. Tubular changes and interstitial exudate were present. In our cases with hypertensive cardiovascular disease no specifically characteristic lesion was demonstrated in the glomerulus by electron microscopy, although a variable degree of glomerular capillary thickening accompanied the arteriolar changes. In the case of abruptio placentae the tubular changes included tubulorhexic lesions.

Comment

One of the challenging problems in the satisfactory understanding of the toxemias of pregnancy has been the prolonged failure to find a single renal lesion acceptable as pathognomonic of this condition. Previous studies, beginning with Löhlein,[6] recognized the glomerulus as the principal site of pathologic change in fatal cases of eclampsia. This author described swelling and increase in capillary endothelium and thickening of the capillary walls, but he interpreted this as resembling glomerulonephritis. Fahr [7] found that in preeclampsia,

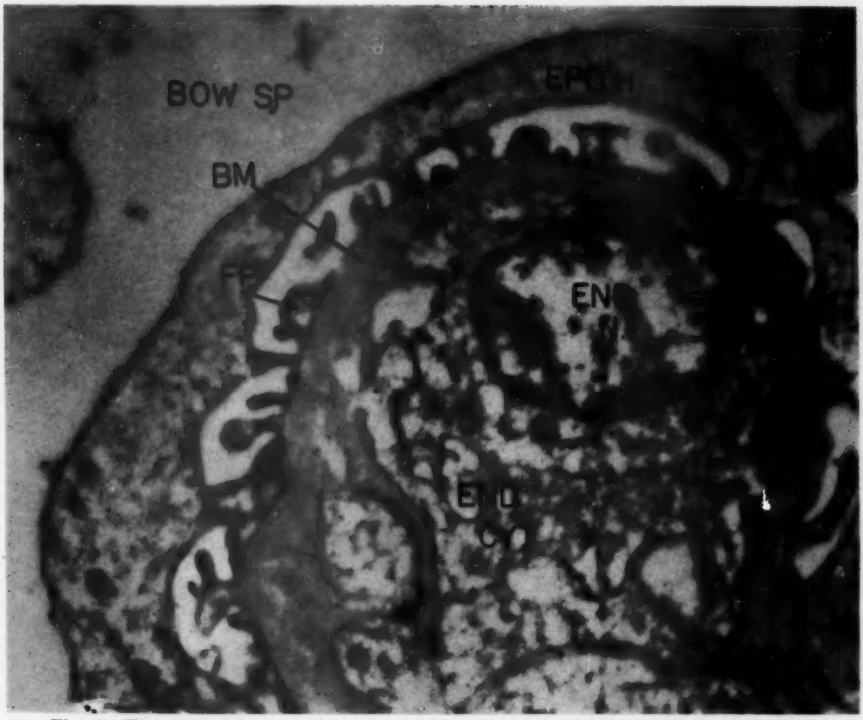
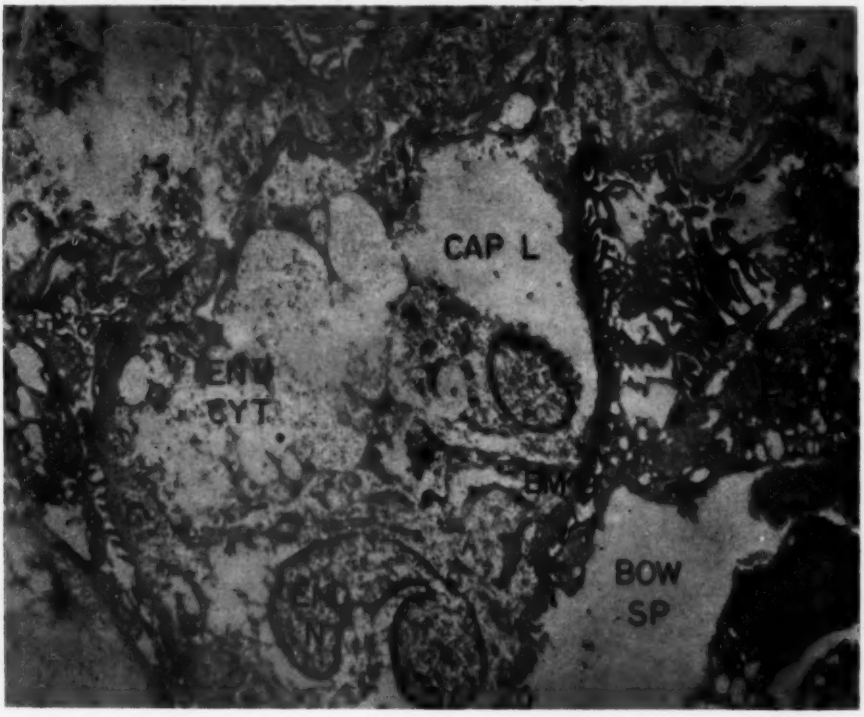


Fig. 4.—Electron microscopy of the same biopsy specimen, showing a glomerular capillary lumen obliterated by endothelial cytoplasmic changes. × 7,500.

Fig. 5.—The biopsy specimen taken 11 days after delivery has many areas in which the endothelial-cell cytoplasm is irregularly vacuolated and the capillary lumen widened.



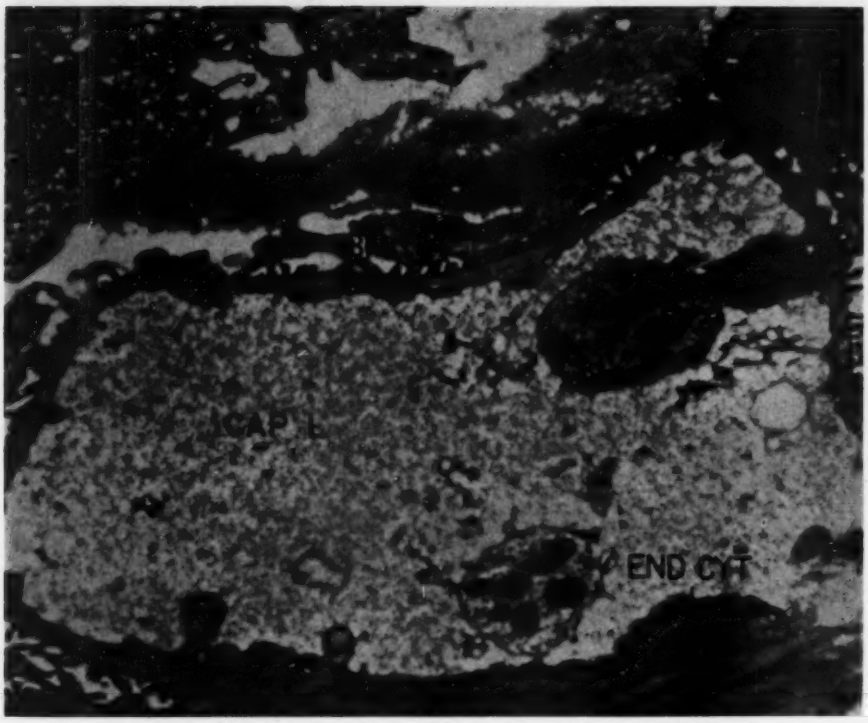


Fig. 6.—Biopsy four weeks following delivery shows a normal capillary lumen.

as well as in eclampsia, the glomerular capillary walls were thickened, with occlusion of the lumen and destruction of endothelial cells. He interpreted these changes as evidence of a degenerative, as opposed to an inflammatory, process. He suggested that the term glomerulonephrosis be applied to this lesion. Bell [8] interpreted the glomerular change as thickening of the basement membrane and swelling of the epithelial cells. Baird and Dunn described glomerular enlargement, with narrowed capillary loops, produced by an increased number or enlargement of endothelial cells. McManus [9] found a fine reticulation and vacuolation of the intercapillary space with extension into adjacent endothelial capillary cells, without thickening of the basement membrane. Sheehan [10] reported change in the glomerular endothelial cells, resulting in swelling and laying down of fine fibrils either under the basement membrane or as a network between the cells. He also found epithelial cells over the loop thickened, with an increase in their cytoplasm. There has, as a result of this, been disagreement about the inclusion of patients with preexisting kidney disease in most series. This difficulty is readily appreciated with experience, and renal changes found in toxemia may be classified pathologically as membranous glomerulonephritis [11] because of the inability to characterize adequately the site of the principal involvement of the glomerular capillary with methods now in use. The adaptation of electron microscopy for the study of renal biopsy tissues permits a differentiation of these glomerular components. As a consequence, several clinical entities which are characterized by early and consistent involvement of specific glomerular components have been separated from this group.[12] Examples of these entities include

lipid nephrosis with epithelial-cell changes, systemic lupus erythematosus with basement membrane and endothelial-cell changes, and amyloidosis with changes largely limited to the basement membrane.

To this growing list of specific entities, we propose the addition of a lesion which occurs most consistently in cases in which a clinical diagnosis of preeclampsia-eclampsia is made. This has a distinctive glomerular capillary endothelial-cell change, involving the cytoplasm most markedly, with a variety of alterations which are interpreted as noninflammatory in nature. It is suggested that the term glomerular endotheliosis be applied to this lesion. The demonstration of this extensive endothelial-cell change conclusively resolves the question as to what actually is responsible for the reduction in the glomerular capillary space. It should enable us to dismiss the previous theories that the basement membrane is thickened, that interstitial cells are narrowing the lumen, or that the epithelial cells are primarily involved. There is the suggestion that the basic process is related to a change in the endothelial-cell cytoplasmic structures. If this proves to be the case, it may be that it could be best studied using the methods successfully employed in renal-tubule pathology.

The demonstration of this endothelial-cell cytoplasmic change contributes little to the understanding of the proteinuria. The thickening of the capillary wall without morphologic evidence of change in the basement membrane or foot processes of the glomerular epithelial cells is added evidence that we cannot attribute the increased permeability to a simple mechanical defect.

This lesion is unique in that it has not been found to this degree in the normal pregnant or nonpregnant woman. Available data indicate that it is peculiar to certain gravid women with hypertension, proteinuria, and edema. These electron microscopic changes are distinctive enough to aid in specific diagnosis and may eventually permit an accurate prognosis. They may enable one to speculate more reliably on the fundamental nature of the process, with the hope of further understanding the pathogenesis of this very puzzling complication of pregnancy.

Department of Pathology, The University of Chicago School of Medicine.

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Effect of Papain on Bone

I. A Histologic, Autoradiographic, and Microradiographic Study on Young Dogs

BENGT ENGFELDT, M.D.; ANDERS HULTH, M.D., and OLLE WESTERBORN, M.D., Uppsala, Sweden

The longitudinal growth of bone is caused by endochondral bone formation in the epiphyseal plate, as opposed to the increase in breadth, which is due to appositional bone growth. A variety of experimental methods are known by which, in particular, this endochondral growth may be disturbed in laboratory animals. Endocrine disorders, such as those following hypophysectomy, thyroidectomy, or administration of cortisone, play an important role.[1] Feeding young laboratory animals on diets which are generally or specifically deficient, such as diets lacking in vitamins or essential amino acids, will also result in retardation of the longitudinal growth. A recent discovery, which has been found to be one of the most rapid and effective agents retarding the endochondral growth, is papain (Hulth, 1958 [2,3]; Hulth and Westerborn [4]).

Papain is a proteolytic enzyme obtained from the latex of the tropical tree Carica papaya. Crude papain, i.e., the impure drug, is a grayish powder with many practical applications in medicine, in the household, and in industry. Papain is also made available in pure double-crystallized form by some chemical laboratories. Its chemical composition has been analyzed in detail, in particular in several papers by Kimmel and Smith.[5]

Previous Investigations

Earlier authors [6-8] have established that crude papain administered intravenously does not affect adult animals, but has a rapid and strong effect on the hyaline cartilage of ears, trachea, and joints of growing rabbits. As early as four hours after injection, the rabbit's ears collapse and hang down ("curled ears"). This effect continues for two to three days. Simultaneous administration of cortisone will heighten and prolong the effect. According to these authors, the papain effect on cartilage is reversible.

Spicer and Bryant's [7] exhaustive histologic studies of ear cartilage after papain injection disclosed, among other things, diminished basophilia of the matrix and a change in metachromasia, which within a period of four hours first increased and then sharply decreased. The chondrocytes at first shriveled, only to swell strongly after four hours, whereas the matrix within the same period of time after the injection first expanded and then decreased in size.

The morphologic effect of crude papain on epiphyseal cartilage in various laboratory animals (young rabbits, cats, mice, rats, and guinea pigs) has been described by Hulth [2,3] and Hulth and Westerborn.[4] They observed that after three hours the proliferating cartilage cells became swollen, while the nuclei showed an irregular chromatin staining. At the same time, the extent of the intercellular matrix was significantly diminished. After 20 hours the cartilage cell columns were irregular, while the bone trabeculae adjoining the epiphyseal cartilage were coarser and more irregular than usual. After a single injection these changes were found to be reversible, so that the epiphyseal cartilage had practically resumed its normal appearance after seven

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From the Department of Pathology II, and the Departments of Orthopaedics and Surgery, University of Uppsala.

days. Even after a single injection, roentgenologic changes were visible in the form of a distinct narrowing of the epiphyseal plate. If repeated injections were given at intervals of one to three days, the damage could become irreversible and the endochondral growth permanently arrested, resulting in bony closure of the epiphyseal lines. Crude papain was used in these experiments.

Using purified papain, Thomas, in his first experiments, failed to obtain any effect. The question of why only crude papain affects cartilage tissue, while the purified preparation is ineffective, appears to have been solved by McCluskey and Thomas[9] in 1958. Papain requires free sulfhydryl groups for activity; i.e., it is active in reduced form. Addition of, for instance, iodoacetic acid, hydrogen peroxide (H_2O_2), or heavy metals, will inactivate papain. Inactive papain can be reactivated by adding cysteine, hydrogen sulfide (H_2S), edathamil (Versene), etc. Crude papain contains both active and inactive papain. McCluskey and Thomas[9] found that only crude papain, or purified papain which had been inactivated by addition of iodoacetic acid, acted upon the cartilage tissue when intravenously injected. If cysteine was added to the crude papain, even this became ineffective. Ingenious in vitro studies provided corroboration of their theory that inactive papain is activated locally in the cartilage, while from the beginning active papain is probably destroyed before it reaches the cartilage.

In the present investigation, we combined histologic, histochemical, autoradiographic, and radiomicroscopic techniques. It was designed to permit a more detailed study of the changes occurring in the zone of proliferation of the cartilage and adjoining parts of the metaphysis after papain injections in growing dogs.

Material and Methods

Young dogs were used for the experiments. Of two litters of five animals each, four dogs each were simultaneously injected with papain, while one animal was used as a control. The dogs of the older litter were about 5 months old, and the average weight of the dogs was about 9 kg. The dogs of the younger litter were about 2.5 months old, and their average weight was about 2.5 kg.

A filtered 2% solution of crude papain in isotonic saline was used. The dogs were given intravenous injections of 10 mg. per kilogram of body weight. The dogs of both litters were killed at intervals of 12, 24, and 48 hours and 7 days after the papain injection. All animals, including the controls, were given sulfur-labeled sodium sulfate intravenously 12 hours before being put to death. The dose for the older dogs was 4 mc. each, and that for the younger animals, 2 mc. each. The fore- and hindlegs of the dead animals were examined roentgenographically. The radius, metacarpal bones, costal epiphyses, tibia, and femur were then removed for further examination.

Using a rotating circular saw with a thin blade, several longitudinal sections of 1-2 mm. thickness, comprising epiphysis, epiphyseal plate, and adjoining parts of the metaphysis, were removed from each bone. A number of different processes were applied to these sections. Some were first embedded in methyl methacrylate and were then ground down to a thickness of approximately 100μ. These ground sections were reserved for contact autoradiography and for radiomicroscopic studies of the distribution of mineral salts. A few sections from each bone were fixed in neutral formalin. Part of those were first decalcified in formic acid and then embedded in paraffin, while the remaining sections were immediately embedded in paraffin without previous decalcification. The paraffin-embedded specimens were cut into sections of 5μ thickness, which were used for histologic and autoradiographic examination, and for studies of the dry weight distribution in tissue, using ultrasoft-roentgen radiation.

In the *histologic examination*, the specimens were stained with Ehrlich's hematoxylin-eosin, Mayer's hemalum-eosin, or Delafield's hematoxylin-eosin method. Even PAS staining was performed, Mayer's hemalum being used as contrast staining.

In the *autoradiographic examination* of the ground specimens Agfa-Printon film was used. The sections were mounted in a special plate holder and covered on both sides with film to ensure good contact between emulsion and specimen. In addition, stripping-film technique (Kodak scientific plates AR-50) was used for autoradiographic examination of 5μ-thick microtome sections after staining with Delafield's hematoxylin.

The *roentgenomicroscopic examination* consisted of two parts. On one hand, ground sections were used for a study of the distribution of mineral salts.[10] The radiation source was a Philips diffraction unit with Cu anode. The specimens were placed directly in contact with an extremely fine-

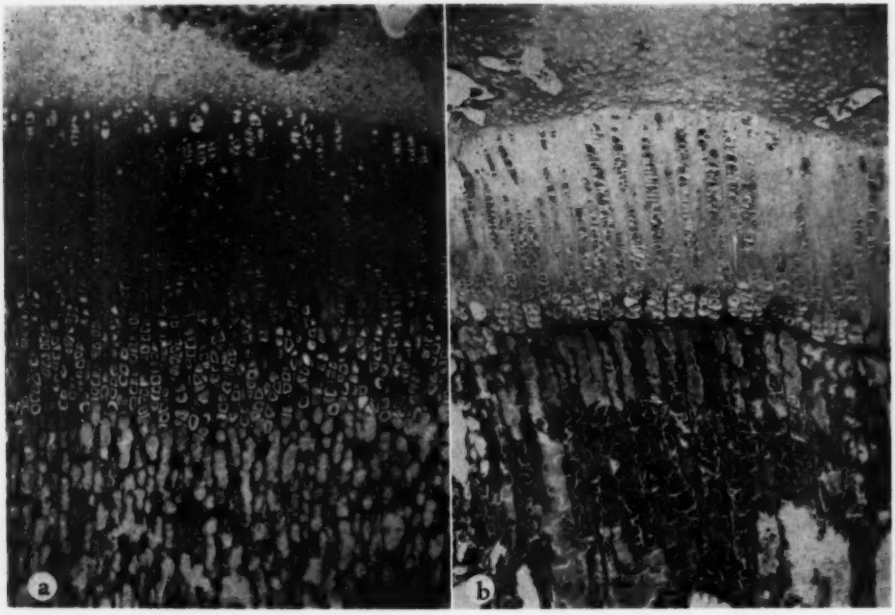
grained photographic emulsion, permitting enlargements of up to 1,000 times. Exposure was made by roentgen rays generated at 24 kv. The pictures obtained in this way were enlarged by photomicrography. In addition, roentgen technique was used to study the distribution of dry mass in paraffin-embedded sections, using the method described by Engström and Lindström,[11] in 1950. The variations in mass per unit area are with this technique made visible as variations in roentgen absorption of different tissue structures. This technique is also based on the principle of secondary magnification.

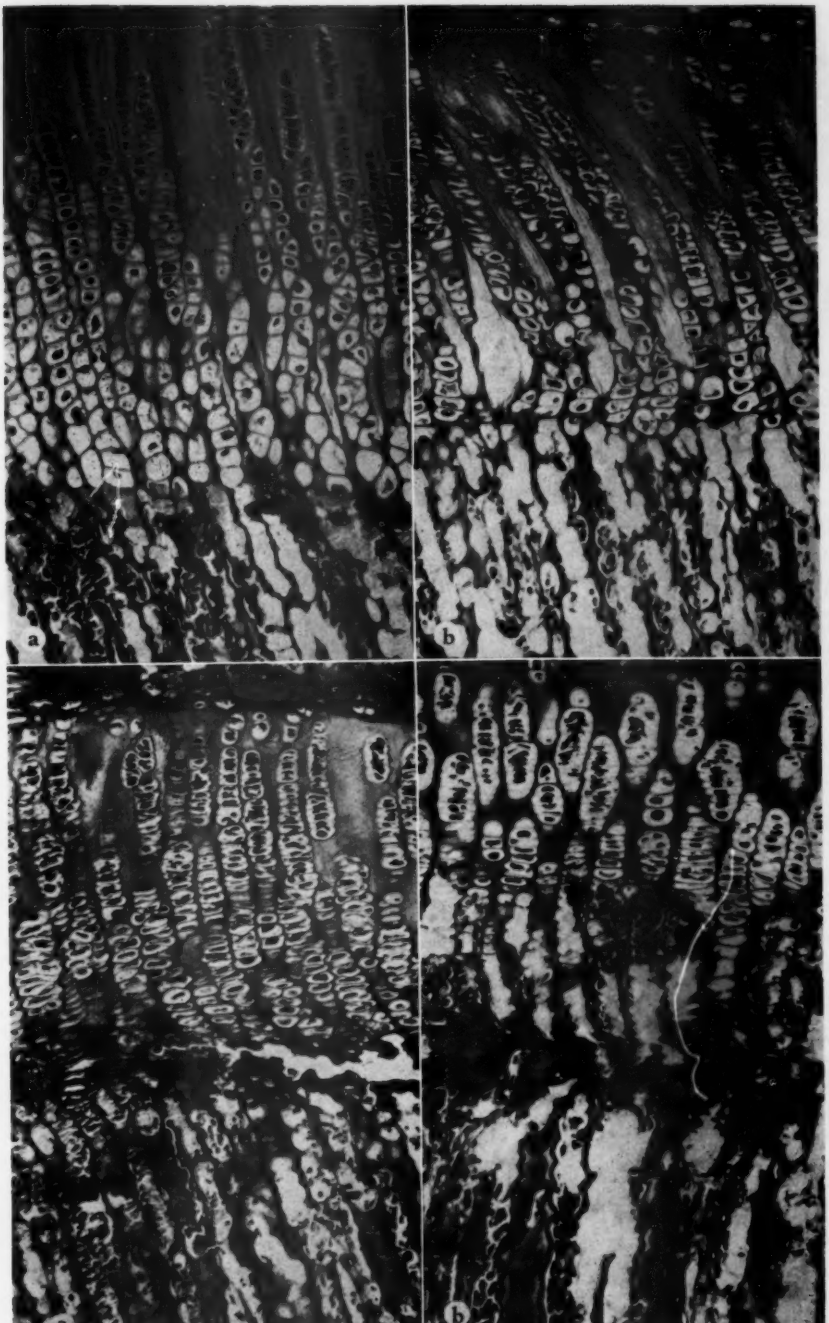
Results

Roentgen Examination.—Clinical roentgen examination 48 hours and 7 days after papain injection disclosed a distinct narrowing of the epiphyseal plate. The changes were most evident in the radius and ulna.

Histologic Examination.—Twelve hours after injection of papain, the matrix of the epiphyseal cartilage was found to have decreased considerably in extent and, furthermore, to have lost its basophilic staining properties (Fig. 1). In addition, the fibers of the matrix were found to be more clearly visible than normal (Fig. 2). The chondrocytes were changed generally, but the changes were least noticeable in the resting cartilage. Changes in the cells seemed to be more extensive the closer these cells were to the metaphysis, and appeared to be most decided in the hypertrophic cells. The nuclei of the proliferative zone, which normally are wedge-formed and stain darkly with hematoxylin and eosin, had become rounded, almost vesicular, and staining was weaker and more irregular. The endoplasm in these cells appeared to have swollen considerably. The hypertrophic cells, which normally are polygonal and light in color, had become compressed and irregular, and appeared to have decreased in number. These changes had become even more noticeable 24 hours after injection, when, in addition, a necrotic zone was visible in the border area between the hypertrophic cells

Fig. 1.—Radius. Smaller litter. The histological changes of the epiphyseal cartilage 12 hours after papain injection are seen in (*b*), as compared with the normal picture in (*a*). Disappearance of the basophilia, narrowing of the epiphyseal plate, and severe damage of the chondrocytes, especially the hypertrophic cells, are obvious. Ehrlich's hematoxylin-eosin stain; reduced to 68% of mag. × 100.





Figs. 2 and 3.—Radius. Larger litter. Figure 2*a* shows the normal epiphyseal cartilage; Figures 2*b* and 3*a* and *b* show the consecutive changes 12, 24, and 48 hours, respectively, after injection. Note the increased visibility of the fibers of the matrix and compression of the zone of hypertrophic cells after 12 hours. The extension of the matrix is diminished from 24 hours. A manifest epiphyseolysis is also seen. Forty-eight hours after injection the bone trabeculae are deformed and broader. PAS-Mayer's hemalum; reduced to 64% of mag. × 256.

and the metaphysis. In nearly all slides, cracking of the specimen through this zone was observed (Fig. 3*a*). It may be supposed that this had at least partly originated intra vitam. Forty-eight hours after injection it appeared as though the chondrocytes had again started to proliferate; but the proliferation was highly irregular, with clustering of the cells (Fig. 3*b*). The cartilage cell columns had at this stage lost their normal regular appearance. In some parts of the epiphyseal cartilage the transverse septa between the hypertrophic cells had disappeared, and the capillaries in these parts seemed, therefore, to proceed to a higher level than in the others (Fig. 3*b*). No hypertrophic cells of normal appearance could be observed; the cells adjoining the metaphysis were almost of a proliferating type. At this stage the changes had progressed down to the metaphysis. The slender trabeculae, closest to the epiphyseal cartilage, had become spiral-shaped or showed a zigzag pattern. The bone trabeculae in the metaphysis adjoining this area were observed to be coarser and more ir-

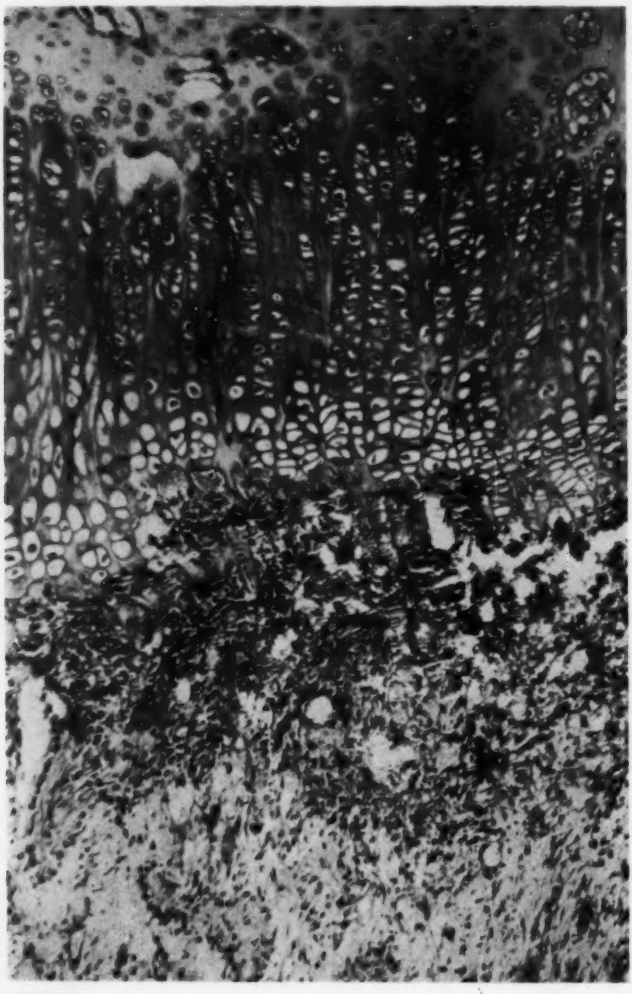


Fig. 4.—Costochondral junction, seven days after injection. Larger litter. The irregular growth zone is demonstrated. A healing epiphyseolysis, with ingrowth of connective tissue and membranous bone formation, is seen. Ehrlich's hematoxylin-eosin stain; ×84.

Fig. 5.—Costochondral junction, 48 hours after injection. Larger litter. The derangement and compression of the hypertrophic cells are well visible. Between the hypertrophic cartilage and the metaphyseal bone trabeculae a zone of necrotic material is evident. Ehrlich's hematoxylin-eosin; approximately ×640.

regular than normal (Figs. 3*b* and 5). Seven days after injection the changes in the bone had increased: Coarse and irregular bone trabeculae were visible spreading down from the hypertrophic cells, which at this stage developed again. In some specimens necrotic areas, alternating with moderately cellular granulation tissue, next to the epiphyseal cartilage were signs that the cracks observed at an earlier stage were healing. Islands of membranous bone formation were visible in this granulation tissue (Fig. 4). Within the epiphyseal cartilage signs of healing were also observed. The basophilia had been restored, and differentiation of cells of varying maturity had again become evident. The cartilage cell columns, however, were still arranged in a highly irregular fashion, in both general organization and length. In some specimens from the younger dogs, seven days after injection an open cleft was visible traversing the epiphyseal cartilage between the proliferative and the hypertrophic zone. In addition, the borderline between epiphysis and metaphysis, which normally is almost straight, was irregular and wavy.

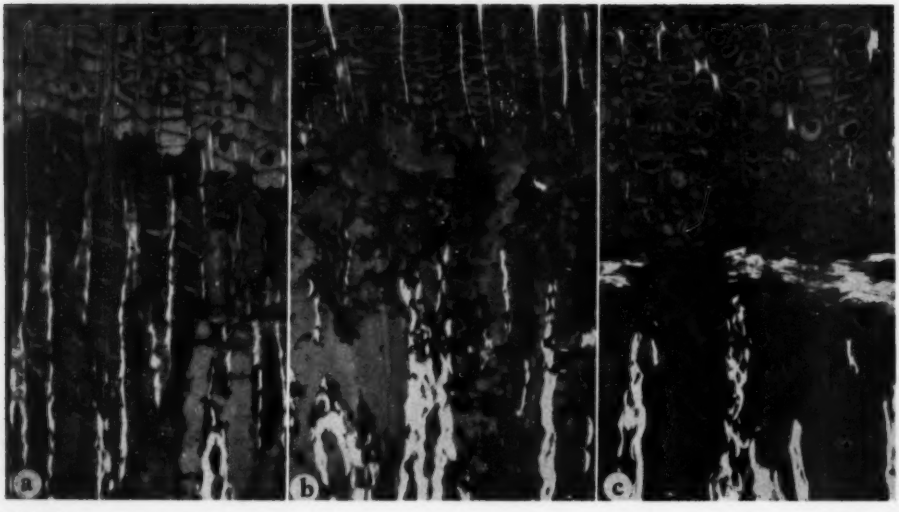
Microscopic Examination Under Polarized Light (Fig. 6*a-c*).—This disclosed typical changes in the organization of the collagen fibers. As early as 12 hours after injection a break in these fibers could be observed corresponding to the necrotic area on the borderline between cartilage and metaphysis. The same condition appeared to have progressed somewhat lower down in the metaphysis. Forty-eight hours after injection the collagen fibers were found to be spiral-shaped within a small area slightly below the epiphyseal cartilage. Seven days after injection transverse collagen fibers could be seen at the borderline between epiphysis and metaphysis in some specimens. These fibers were lying in the islands of granulation tissue, which at this stage could be observed in those areas.

Soft-Roentgen-Ray Examination for Determination of Dry Weight.—Soft-roentgen-ray technique (Fig. 7 *a*, *b*) confirmed the occurrence of the histologic changes described in the foregoing sentences. The decreased extent of the matrix was clearly visible, and its increased fibrous appearance was evident. The proliferative cells looked considerably swollen, while the wrinkled and compressed appearance of the hypertrophic cells was even more noticeable than in the histologic specimens. As compared with the normal specimens, these pictures showed, 12 and 24 hours after papain injection, a clearly visible difference in roentgen absorption between the parts of the epiphyseal cartilage. This is explained by the fact that the resting cartilage closest to the bony nucleus of the epiphysis in the papain-treated specimens has a relatively slightly higher dry weight per unit area. The necrosis in the border area between epiphyseal cartilage and metaphysis, observed in the histologic examination, was in this examination visible as a zone of higher roentgen density, i.e., a zone with a higher dry weight per unit area.

Soft-Roentgen-Ray Examination for Determination of Mineral Salts.—With this method only the mineralized structures are made visible. It is somewhat difficult in

Fig. 6.—Radius. Smaller litter. Polarized light microscopy (× 180), in which the collagen bundles are seen as light areas running from the cartilage down in the metaphyseal bone trabeculae.

(*a*) Control specimen; (*b*) changes 24 hours after papain injection are seen. The collagen bundles are interrupted at the bone-cartilage junction. After 48 hours (*c*) epiphyseolysis with development of transversely running collagen fibers is seen. Reduced to three-fourths of original size.



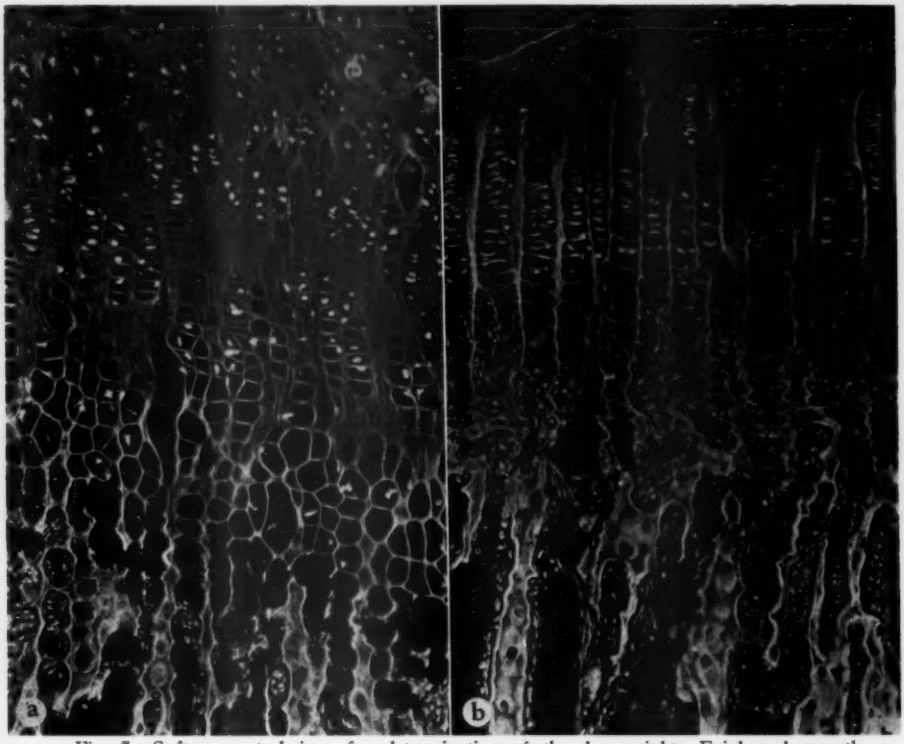


Fig. 7.—Soft-x-ray technique for determination of the dry weight. Epiphyseal growth zone of metatarsal bones.

(*a*) Control; (*b*) changes 48 hours after papain. Light areas have a higher dry weight per unit area than dark ones. The extension of the matrix is diminished. The trabeculae show an irregular zigzag pattern, and the transverse bars at the hypertrophic zone are often broken. Reduced to 74% of mag. × 256.

such specimens to see the exact border between epiphyseal cartilage and metaphysis, i.e., the place where the hypertrophic cells are ready for penetration by the ingrowing capillaries. This is because the matrix between the lowest hypertrophic cells is calcified. The epiphyseal cartilage absorbs only insignificant amounts of roentgen rays of the quality used with this method, and therefore shows up quite black (Fig. 8*a*). In 24 to 48 hours after injection with papain, however, this black zone was found to be narrowed, as compared with the normal specimens. In 12 to 24 hours after papain injection a transverse zone of higher roentgen density was observed across the mineralized columns. This zone may be thought to correspond to the necrosis observed in the histologic examination (Fig. 8*b*, *c*). Close to this zone, cracks similar to those observed in the histologic examination readily occurred, providing helpful orientation for comparison with the histologic specimens. The zone of higher roentgen density could be seen to have shifted gradually down to the metaphysis in the interval between 12 and 48 hours after injection, as a sign that some growth had occurred during this time. The changes which could be observed 48 hours after injection were very typical; i.e., in most of the specimens lateral displacement and angulation of all mineralized columns were visible, combined with an increased roentgen absorption (Fig. 9*a*, *b*). This change probably occurred during the

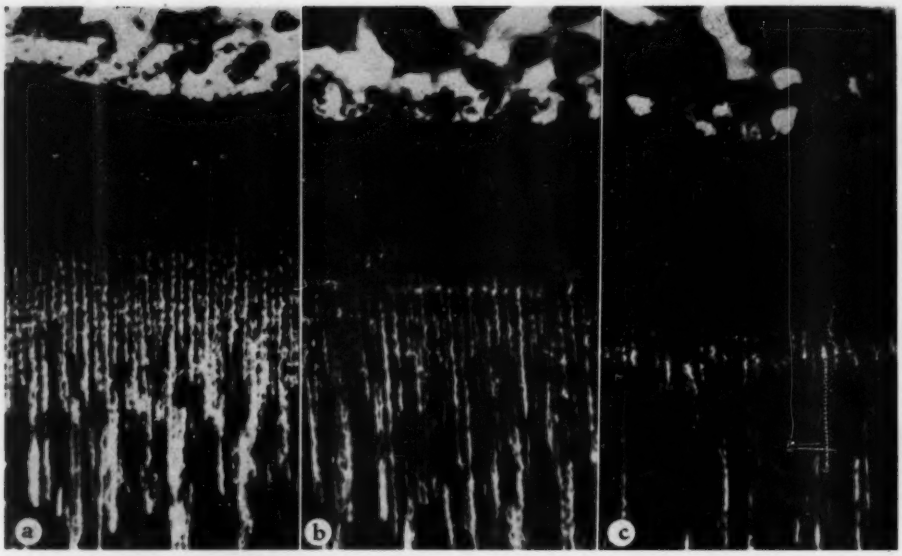


Fig. 8.—Radius. Small litter. Soft-x-ray technique for demonstrating mineral salts in ground sections (light areas).

(*a*) Control specimen, showing the straight mineralized trabeculae (light areas) proceeding down from the epiphyseal cartilage (black). (*b*) Twenty-four hours after injection of papain a break of the trabeculae corresponding to the epiphyseolysis is visible. A zone of higher density beneath the epiphyseolysis has in (*c*) (48 hours after injection) been shifted downward to the metaphysis. Reduced to 69% of mag. × 84.

short period of time in which the damaging effects of the papain injection caused the growth to be most retarded. Seven days after papain injection the columns were observed to be considerably shorter and more clustered than normal in all specimens, but especially in those taken from the older litter. In some specimens a denser zone across the trabeculae was visible even at this stage, but it had now moved a considerable distance down the metaphysis.

Investigation with Sulfur-Labeled Sodium Sulfate.—The autoradiographs of ground sections invariably showed a high uptake of S^{35} in the epiphyseal cartilage. In the autoradiographs of normal specimens this showed up as an even and diffuse blackening without any specific pattern. Autoradiographs of the epiphyseal line 12 hours after papain injection, however, showed a lighter zone lying like a ribbon across it, and covering approximately one-fourth the height of the labeled area. The localization of this lighter zone corresponded to the lowest rows of proliferating cells and the upper rows of hypertrophic cells. This lighter zone could not be explained by changes in the histologic picture. There were no histologic changes restricted to this part of the epiphyseal cartilage. The part of the epiphyseal cartilage adjoining the metaphysis had possibly a slightly larger uptake than the remaining part. Twenty-four hours after injection the labeled area appeared to be unchanged in height, while the lighter zone had shifted and now covered a wide area adjoining the metaphysis (Fig. 10*a-c*).

Forty-eight hours after injection the area of activity was seen to be considerably narrowed. A diffuse blackening was visible, and no lighter zone could be discovered. The autoradiographs seven days after papain were almost identical with those of the normal specimens except for the irregular and wavy appearance of the borderline between the labeled area and the nonradioactive zone (Fig. 10*d*).

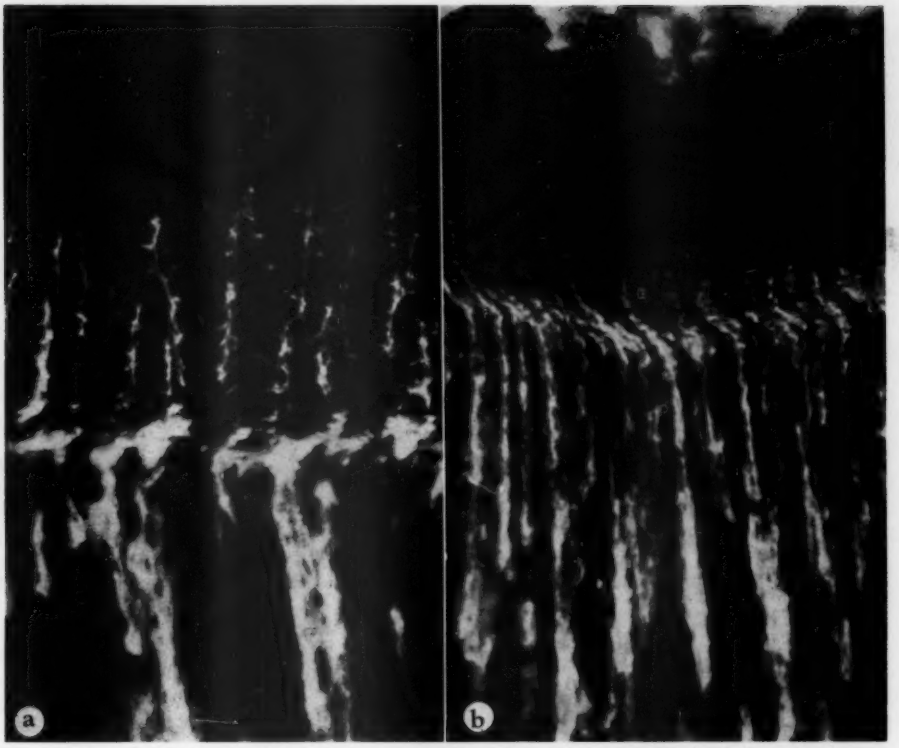


Fig. 9.—Soft-x-ray technique for demonstrating mineral salts in ground sections, showing the changes of the mineralized trabeculae of costal (*a*) and metatarsal (*b*) metaphyses 48 hours after injection. Reduced to ¾ of mag. × 256.

The autoradiographs made with stripping-film technique of paraffin-embedded sections showed a noticeable uptake of S^{35} in the epiphyseal cartilage. In the normal specimens the blackening had a distinct columnar pattern in the decalcified sections. This pattern would seem to be correlated with the normal columnar arrangement of the cartilage cells. The columnar pattern disappeared after papain treatment, and the diffuse distribution could be seen clearly after only 12 hours (Fig. 11*a*, *b*). Seven days after papain injection, the photographic blackening was again seen to have a columnar pattern, although it was somewhat less regular than usual.

It was not possible to determine accurately whether the sulfur was located in or outside the chondrocytes. According to Belanger,[12] it would seem to be located intracellularly, a localization which may explain the columnar pattern in normal specimens. In normal specimens the photographic blackening covered the entire epiphyseal cartilage down to the border of the metaphysis. Twelve hours after papain treatment a clearly marked zone of higher concentration was visible at the border of the metaphysis. Forty-eight hours after injection the area of hypertrophic cells adjoining the metaphysis showed no uptake of sulfur (Fig. 11*c*).

Comment

Papain appears to have a relatively selective effect on cartilage tissue in all growing animals, but does not affect the cartilage of adult animals. The effect is most noticeable

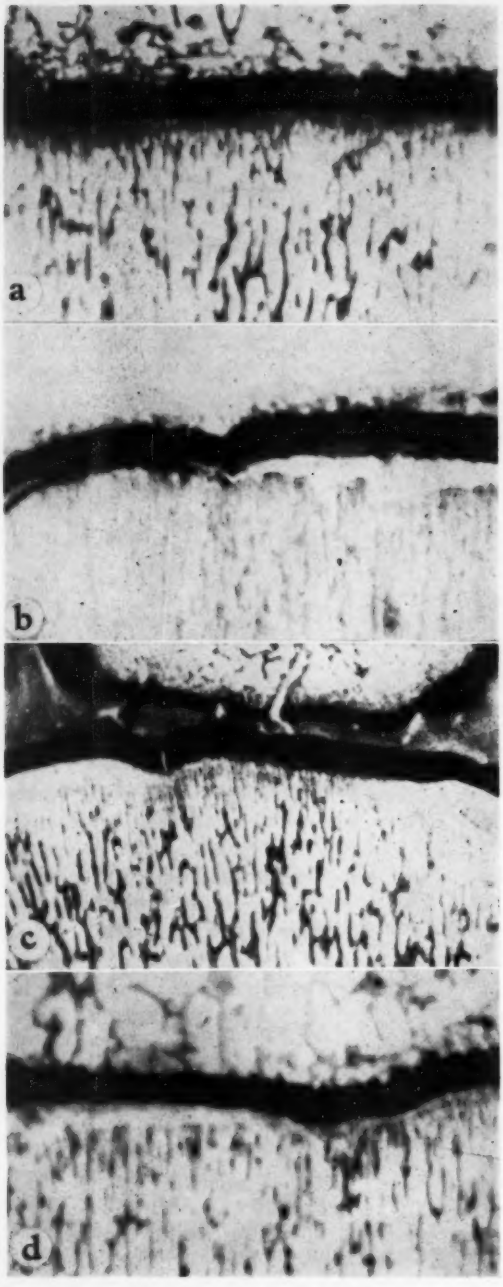


Fig. 10. — Autoradiograms obtained from ground sections. (Agfa-Printon film). Radius of the smaller lit.er. × 13.

(*a*) Normal pattern of uptake of radioactive sulfate. Note the diffuse uptake of radioactivity over the entire epiphyseal cartilage.

(*b*) Twelve hours after papain injection. A lighter zone lying like a ribbon across the epiphyseal cartilage and covering about one-fourth of the epiphyseal height is seen.

(*c*) Twenty-four hours after papain. The lighter zone is now covering a part of the epiphyseal cartilage close to the metaphysis.

(*d*) Forty-eight hours after papain. The labeled zone is narrowed and more irregular than above, but no lighter zone is seen at this moment.

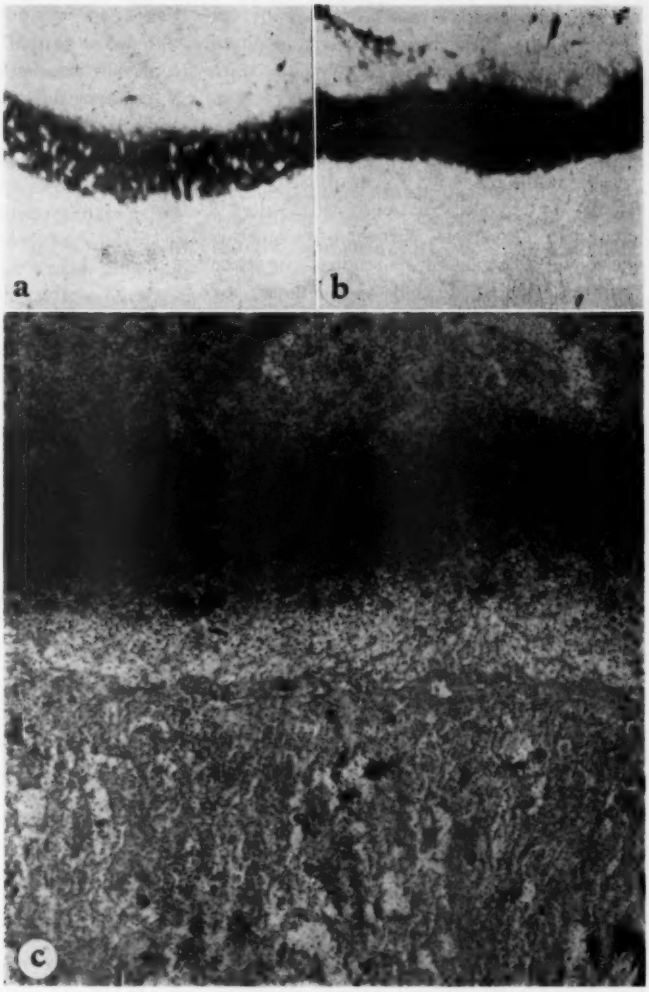


Fig. 11. — Autoradiograms obtained with stripping film.

(*a*) Normal pattern, with a definite tendency to columnar shape, of the uptake of radioactive sulfate of the epiphyseal plate. Radius. × 32.

(*b*) Twelve hours after papain injection the uptake seems to be diffusely distributed. Radius. × 32.

(*c*) Forty-eight hours after papain a diffuse uptake, leaving the zone of hypertrophic cells close to the metaphysis without any appreciable uptake.

in the epiphyseal cartilage, particularly in its actively growing parts, i.e., the proliferating and hypertrophic zones. Of interest is the fact that the morphologic changes in the epiphyseal cartilage develop extremely rapidly and, according to earlier investigations, are visible already three hours after injection. The effect of repeated treatment with papain can, as mentioned in the foregoing observations, cause permanent damage, resulting in bony closure of the epiphyseal cartilage. The resting part of the epiphyseal cartilage appears to suffer least from papain injection. From this zone down to the metaphysis, however, the changes are seen to become progressively more advanced, so that the hypertrophic cells adjoining the metaphysis are damaged most. In the metaphysis nearest these cells, cartilage septa and collagen fibers even show a decided tendency to become necrotized. In addition, the entire epiphyseal cartilage loses its basophilic staining properties, while the fibrous appearance of the matrix is more evident than usual. This is thought to be caused by a

release and disappearance of part of the chondroitin sulfate, probably as a result of the papain acting on the proteins to which the chondroitin sulfate is linked. Examination with radiosulfate provides corroboration for such an assumption. Previous investigations made by Spicer and Bryant [8]; Bryant, Leder, and Stetten,[13] and Tsaltas [14] have also served to throw light on this question. The chemical studies of those authors established that the amount of chondroitin sulfate in blood and urine increases after papain injection,[13] whereas it simultaneously decreases in the cartilage.[14] The necrosis observed in the border area between epiphyseal cartilage and metaphysis is, 12 to 24 hours after papain, often accompanied by cracking of the specimen in or near the necrotic zone. It can be discussed whether this cracking is to be regarded as an artifact or as an epiphyseolysis originating intra vitam. However, since it is encountered almost uniformly in all specimens, it seems reasonable to regard the cracking as the result of a locus minoris resistentiae conditioned by the necrosis. The assumption that the cracking has at least partly started intra vitam is further strengthened by the fact that seven days after papain injection some specimens show signs of healing in the form of ingrowth of connective tissue, and even membranous bone formation. The histologic picture closely resembles that of a callus. Why a necrosis should occur just in the border area between epiphyseal cartilage and metaphysis is an undecided question. This localization might be explained by the fact that just in this area the hypertrophic cells open for penetration by the ingrowing capillaries. It is plausible that the papain leaves the blood stream via these capillaries and from this point begins its attack on the proteins in the matrix, with, as it were, maximum effect. Papain is, after all, a proteolytic enzyme, which may be supposed to affect not only the proteins linked to chondroitin sulfate but also other proteins, such as collagen. In the border area between epiphysis and metaphysis the very thin trabeculae afford the papain an opportunity to attack the collagen fibers, causing a break in the collagen trabeculae and possibly leading to an epiphyseolysis.

Papain does not appear to cause any serious changes of the mineralization of the trabecular system. The damage is highly localized and corresponds to the necrosis and the break in the collagen fibers observed in the histologic specimens. The trabeculae in this area are seen as a zone of higher roentgen density. This change is seen to have moved down the metaphysis in the intervals between 12-24 and 24-48 hours after papain injection. At the latter stage, typical changes are visible in the trabeculae somewhat below the border of the metaphysis, the trabeculae having become laterally displaced and angulated. At the same time, the collagen fibers are found to be spiral-shaped. This disturbance in the mineralization is probably caused by the necrosis and compression of the hypertrophic zone, but may also to some extent be indicative that the growth has been arrested for a short time. Otherwise the growth seems to have proceeded in the intervals checked, since the localized changes are found to have moved down in the interval between 12 and 48 hours after papain injection.

Evidence of the changes described in the foregoing paragraph has also been provided by investigation with radiosulfate. According to Belanger [12] and Amprino,[15] the sulfate will normally have accumulated in the cartilage cells within one or two hours after injection, and will then gradually pass into the matrix. These authors state that after 24-48 hours a diffuse concentration of sulfate will be found in both cells and matrix. At a later stage practically all sulfur is located in the matrix. On this observation Belanger [12] has based his assumption that chondroitin sulfate is formed in and by the chondrocytes. The lighter zone described in the autoradiographic observations, lying across the epiphyseal cartilage,

may be an indication of decreased synthesis of chondroitin sulfate in this area but may, on the other hand, be due to a release of chondroitin sulfate synthetized in those 12 hours. This, as well as the disappearance of the columnar uptake of radioactivity in stripping autoradiographs, could perhaps be explained by swelling of the chondrocytes, by damage to the permeability, or by the general derangement of the cellular columns. The downward movement of the lighter zone in the interval between 12 and 24 hours after papain injection may indicate that the renewed proliferation of the chondrocytes is pushing the damaged area downward, but other explanations are possible.

Further studies are in progress on the effect of papain on the sulfate uptake in epiphyseal cartilage. These studies may shed more light on the events occurring in sulfur metabolism under normal and pathologic conditions.

The present investigation has been helpful in elucidating the morphologic effect of papain injection. Nevertheless, there are still many interesting questions which have remained unanswered, in particular the question why papain has such a selective effect on growing cartilage only and does not affect the cartilage of full-grown animals. The effect of papain on cartilage is most noticeable in the epiphyseal cartilage, in particular, in its most active parts, i.e., the proliferating and hypertrophic cell areas. From an earlier investigation [16] it is known that papain affects adult rabbits in that it causes necrosis of the muscle fibers, in particular in actively working muscles, such as those of heart and diaphragm. This, together with the interesting phenomenon that papain apparently must be activated *in loco* to produce any effect in vivo, could be an indication that papain in some way interferes with the normal function of the enzymes involved in cartilage growth and muscle activity.

Summary

The strong effect of crude papain on epiphyseal cartilage of growing dogs is described.

Histologic examination, which included polarization microscopy, disclosed considerable damage of the proliferating and hypertrophic cells. Even the matrix was altered and had lost its basophilia. The entire epiphyseal cartilage was narrowed. Necrosis and epiphyseolysis were evident in the border area between the epiphysis and the metaphysis. A break of the collagen fibers in this zone could be observed.

Soft-x-ray technique provided evidence of localized damage of the mineralized trabeculae.

Autoradiography with radioactive sulfate showed a diminished uptake in a transverse zone of the epiphyseal cartilage 12 hours after papain injection.

The above-mentioned changes all appeared in the short interval of from three or four hours to seven days after a single injection of crude papain.

Department of Pathology II, University of Uppsala.

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Demonstration of the Marrow—Vascular Space (Macrocanalicular System) of Bone

Technique for Production of Three-Dimensional Plastic Anatomic Models

LLOYD A. HURLEY, M.D., New York, and CLARENCE W. MILLER, M.S., Bethesda, Md.

Introduction

Contrary to general belief, bone is a relatively vascular tissue, more so, for instance, than adipose or fibrous connective tissue. Diffusion of nutrients through the mineralized ground substance of bone is much more difficult than in any other tissue of the body. Measurements made on a dog's radius (Ham,[2] 1952) have shown that bone cells, if they are to survive, can generally be no farther than 100μ from a nutrient vessel. Nature has overcome this particular problem of ossified tissue by developing within such tissue a unique vascular network. The configuration of the channels through which these vessels pass in different types of bone is quite varied and most interesting. In this laboratory attempts to study the minute vasculature of bone by commonly used injection techniques[4,7,8] have been unsuccessful, owing to the extremely small diameter of the vessels occupying the Haversian and Volkmann canals (macrocanalicular system).

A technique has been developed in which a plastic model of these tunnels can be prepared. This is accomplished by utilizing bone extracted with ethylenediamine ("anorganic bone").[3]

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Biologist, Dental Division, Navy Medical Research Institute, Bethesda, Md. (Mr. Miller).

Orthopedic Research Laboratory, Columbia University College of Physicians and Surgeons, New York, and Navy Medical Research Institute, Bethesda, Md.

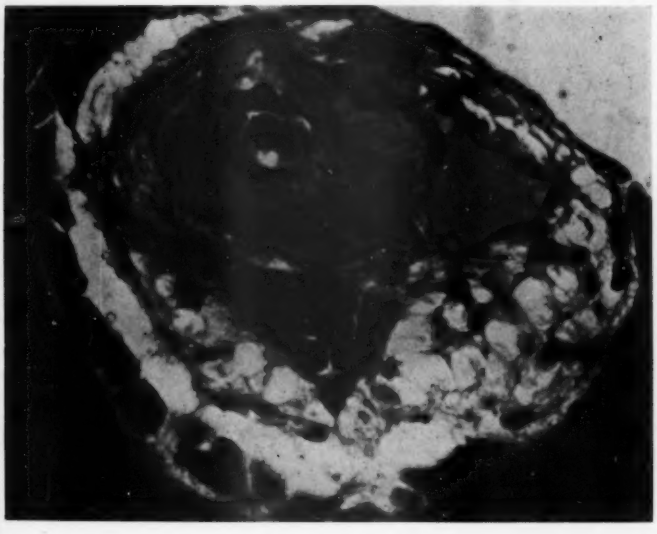


Fig. 1.—Photomicrograph of an osteon, showing formed elements within a Haversian canal. Note the presence of an arteriole (thick-walled vessel), venule (containing red cells), lymphatic vessel (clear lumen), and cellular marrow elements. Reduced 88% of mag. $\times$ 475.

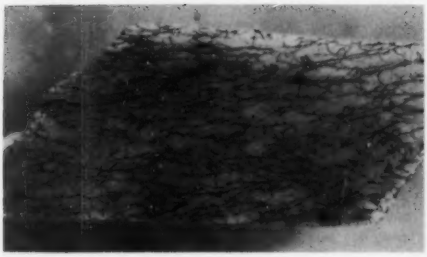


Figure 2

Fig. 2.—Macrophotograph, low power, illustrating macrocanalicular structure of bone. Bovine rib extracted with ethylenediamine. Spaces have been infused with colored vinyl acetate. The specimen was then embedded in a clear methyl methacrylate plastic block.

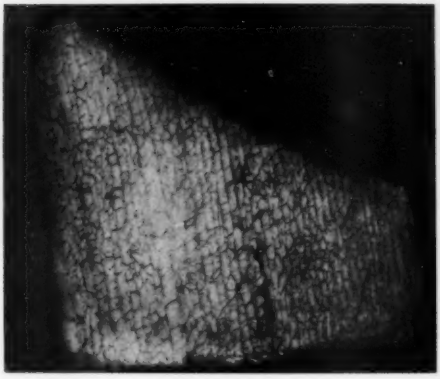


Figure 3

Fig. 3.—Macrophotograph, low power. Dense cortical bone from central portion of cow femur; periosteal surface (left); endosteal surface (right). Note the lamination and the compact organization of the marrow vascular spaces. Observe the gross difference in angioarchitecture of the femur, a weight-bearing bone, and that of the rib, a relatively non-weight-bearing bone. This specimen was also embedded in a clear plastic block and photographed at a later date.

Included within bone, but not considered as ground substance of bone tissue, are the contents of the marrow-vascular space (Fig. 1), consisting of organic matter and including (1) blood vessels, arterial, capillary, and venous, (2) lymph capillaries, (3) the medullary space with the macrocanalicular system.

Figure 4

Fig. 4.—Macrophotograph, low power, illustrating the macrocanalicular structure of bovine ulna. Observe the presence of numerous thin-walled Haversian systems connected by delicate Volkmann canals. The presence of numerous club-shaped blind pockets appears to be a unique feature of bovine ulna.

Figure 5

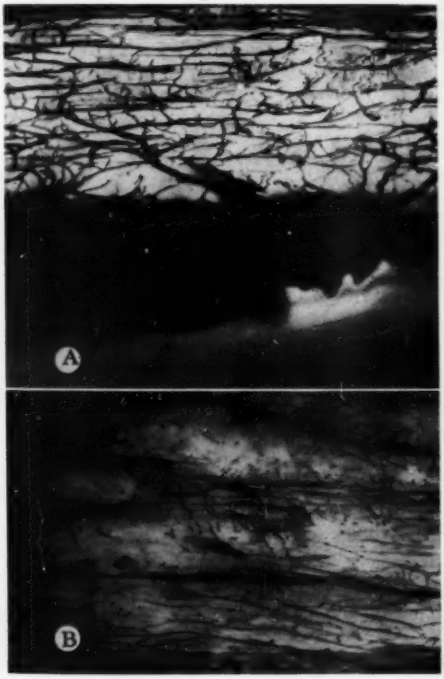


Fig. 5.—*A*, macrophotograph, medium power. Longitudinal section through canine tibia. The communication of the medullary canal (bottom half) with the periosteal surface (top) via an intricate system of branching canals is demonstrated.

B, macrophotograph, medium power. Endosteal surface of canine tibia viewed from within medullary canal. Note the numerous tubules connecting

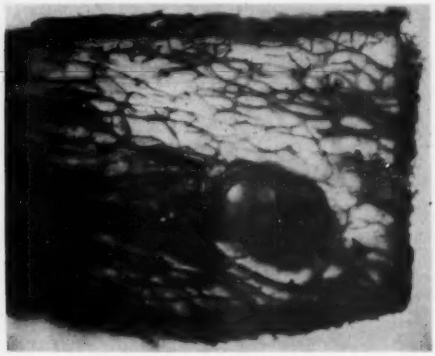
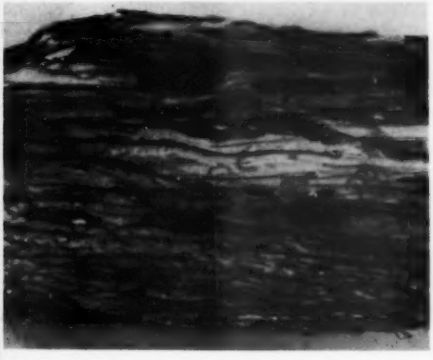


Fig. 6.—Macrophotograph, medium power, human mandible. Section taken around the mental foramen. Note that the longitudinal organization of Haversian and Volkmann canals, as found in long bones, is not present in the mandible. Anastomoses between Haversian systems are numerous and large in many cases, resulting in stellate formation.

nerves, and (4) bone marrow, with its many cellular components. These are the elements removed by extraction of bone with ethylenediamine. The space remaining after their removal consists of a three-dimensional anatomical unit—the macrocanalicular system. If extracted bone is perfused with a colored vinyl acetate or methyl methacrylate plastic, a remarkable reduplication of these spaces can be produced (Figs. 2-7). If the crystalline material of bone is then removed by chelation, a unique three-dimensional model is available for study. We feel that the technique herein described can be utilized as an approach to the basic study of the morphology of normal and diseased bone.

Fig. 7.—Macrophotograph, medium power. Paget's disease, human radius. Note the relatively wide Haversian spaces. The anastomoses between systems are large. The junction between Volkmann's canals and Haversian systems are rounded, as compared with sharp, right-angled anastomoses found in normal bone. This indicates erosion of bone matrix, due to pathological process of this disease.



Methods and Materials

Ethylenediamine Extraction.—Bone samples to be studied were first treated with ethylenediamine according to the method of Williams and Irving,[4,7] as modified by Losee.[5] The effectiveness of ethylenediamine as a solvent for organic material is demonstrated in Figure 8. This effect was more efficiently utilized by using a Soxhlet extraction apparatus (Fig. 9). The constant boiling point of 80% ethylenediamine in distilled water is 117.5-118.5 C. The time for extraction was 32-36 hours, utilizing 150 ml. of ethylenediamine solution. After extraction, the bone was rinsed with distilled water and either air-dried or dried in vacuum.

Perfusion Technique.—Dry-bone samples were placed in test tubes, which were capped with Silicon-rubber stoppers. Using a No. 22-gauge needle to penetrate the silicon-rubber, a vacuum within the tube of approximately 0.5μ to 1μ was effected with a standard Magavac pump. The silicon-rubber stoppers were self-sealing upon removal of the needle from the vacuum source. An 80% mixture of colored vinyl acetate-acetone solution was then injected

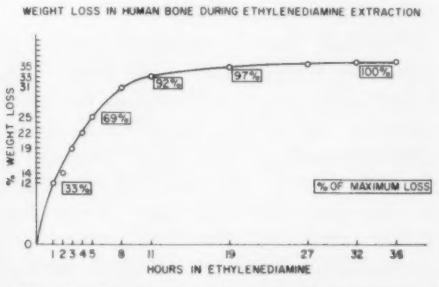


Fig. 8.—Graph illustrating the effectiveness of ethylenediamine as a solvent for organic material in bone. The weight loss in human bone during ethylenediamine extraction is illustrated.

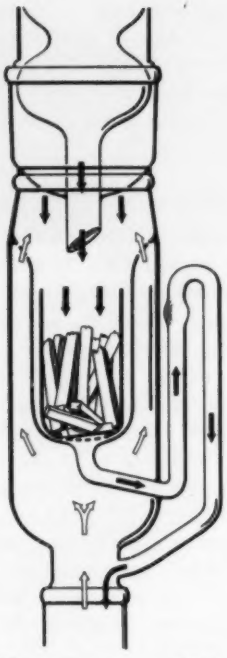


Fig. 9.—Diagram of the modified Soxhlet extractor used in treating bone with ethylenediamine. Samples were kept within 1 degree (C) of the temperature of the cycling liquid, since the vapors of the boiling liquids surround the inner chamber.

with a syringe and a needle through the silicon-rubber stopper in sufficient quantity to cover the bone specimen. It was observed to fill the interstices of the bone preparation rapidly. The silicon-rubber caps were then removed, and the plastic was allowed to solidify (24 hours). The bone specimen was then dissected free of the plastic with forceps and a sharp scalpel.

Chelation and Clearing.—Complete or partial demineralization, accomplished with either a 5% nitric acid solution for rapid chelation or 5% aqueous solution of edathamil sodium (Disodium Versenate)-formalin (5.5 cc. edathamil sodium to 100 cc. of 10% formalin) for slower but gentler chelation. The progress of demineralization could be observed with a low-power dissecting microscope, the structure of the macrocanalicular system becoming more apparent from the surface to the central area of the bone as chelation progressed. Since vinyl acetate is soluble in organic solvents, clearing agents commonly used, such as xylene, chloroform, benzine, or methylsalicylate, destroyed the specimens. It was found best first to dehydrate the specimens to absolute ethyl alcohol and then, depending upon the degree of demineralization, clear them, using either polyethylene glycol 400, if chelation was complete, or cedar oil if, because of incomplete chelation, specimens needed more effective clearing. The specimens could then be kept in these agents for study and for photomicrography. The better preparations were preserved permanently by embedding in clear plastic blocks.

Of the techniques for embedding biologic material in clear plastic, polymerized while surrounding objects suspended in it,[6] we chose to use Selectron No. 5026, an unsaturated polyester resin, as the embedding material. The ingredients are 100% reactive; no toxic by-product is given off in the reaction, and the laboratory operation involved is simple. Selectron was mixed with an oxidizing catalyst (*t*-butyl hydroperoxide) in the proportion of 0.5 ml. of catalyst to 100 ml. of plastic and stirred vigorously. The plastic was then placed in a warming oven at 37 C for 30 minutes, to decrease viscosity, and transferred to a vacuum desiccator to remove air bubbles. A negative pressure of 25-60 mm. of mercury was then applied. The evacuated plastic was poured as a thin layer into a dish and allowed to solidify. Four hours later the bone preparations were placed on the solidified plastic. This procedure prevented the complication of the specimen sinking to the bottom surface of a plastic block. Upon removal from the dish, the plastic blocks were trimmed with a saw. A clear, lustrous finish was produced by buffing with a series of abrasive compounds on a power-driven wheel. The plastic block was first worked with wet pumice on a cloth wheel and then polished by buffing with jeweler's rouge on a chamois wheel. These preparations were

Fig. 10.—Plastic block containing specimen infused with vinyl acetate. The preparations were very durable and could be used indefinitely.

quite durable and could be used indefinitely (Fig. 10). A list of special materials and equipment required for this technique is given in the Table.

Equipment and Supplies

Equipment	Amount
Soxhlet extraction apparatus (Fig. 4)	1
Vacuum desiccator jar with vacuum rubber tubing	1
Megavac high-vacuum pump	1
Power-driven buffing head with 6-in. buffing wheels (1 cloth wheel, 1 chamois)	1
Power-driven disk or belt sander	1
Beakers, 500, 250, and 50 ml	3
Biological dissecting kit, comprising scissors, scalpels, dissecting needles, forceps (pincer type), mosquito hemostats	1
Glass syringes, 20, 10, 5 cc	3
Needles BD, 20-gauge	12
Silicon-rubber stoppers	4
Test tubes, 10 cc	4
Dissecting microscope	1
Miscellaneous laboratory equipment, including hammer, spatula, wire, files, sandpaper, bottles, etc.	

Supplies	Amount
Selectron No. 5026 plastic (Pittsburgh Plate Glass Company, Pittsburgh). Price, $9.00/gal	2 gal.
Oxidizing catalyst, *t*-butyl hydroperoxide (Lucidol Division, Wallace and Tiernan, Inc., 1740 Military Rd., Buffalo 5). Price, $4.30/lb	1 lb.
Vinylite injection solution, red, blue, yellow (Gordon Lacey Chemical Products, Inc., Maspeth, N.Y., or Turtox Biological Supply). Price $4.00/qt., each	2 pts.
Pumice flour, N.F. Octagon Process, Inc., 15 Bank St., Staten Island, N.Y.	1 box
Dental whiting, Conray Products Co., New York	1 box
Hydrochloric acid 35 %	5 lbs.
Acetone (Merck & Co., Inc.)	5 lbs.
Jeweler's rouge	1 stick
Ethylenediamine—$NH_2CH_2CH_2NH_2$—85-88 % (Eastman Kodak Co., Rochester, N.Y.)	1 lb.
Nitric acid	1 lb.
Versene (calcium-disodium) (Refined Products Corp., Lyndhurst, N.J.)	1 lb.
Alcohol, 95%	1 lb.
Polyethylene glycol 400 (Fisher Chemical Co., New York)	1 lb.
Cedar oil	1 lb.

Comment

It must be emphasized that preparations achieved by the technique described do not represent a single vascular system, arterial or venous. They represent, instead, the passageway or tunnel system through which vascular structures communicate in bone. The term "marrow-vascular" includes, by definition, the marrow cavity of bone and the extensions of marrow into the numerous Haversian and Volkmann canals. The concept of the "macrocanalicular system," on the other hand, does not include the central marrow cavity but refers specifically to that system in mineralized tissue which is made up of Haversian and anastomosing Volkmann spaces. The macrocanalicular and the canalicular systems constitute the link between the central marrow cavity and the individual cell of any ossified structure. As is seen in Figure 1, parts of the macrocanalicular system may be found to contain arterial, venular, lymphatic, and cellular marrow elements. Usually, however, a single capillary is the only vascular structure found. The relative distribution of the vascular structures in various areas of these channels remains to be explored. The communication of literally thousands of these tubes from the central marrow cavity with the periosteal surface of bone, as shown in Figure 5*A*, and *B*, may explain such phenomena as very rapid venous drainage of the bone, or of pain associated in osteomyelitis with increased intramedullary venous pressure. Clinically, pain in the bone affected by osteomyelitis is produced by coughing or sighing (Valsalva's maneuver).

Each particular bone in the body has a special mechanical function to perform. As function determines structure, it follows that each bone in the body must have its own unique architectural variation. A study of the normal variation in architecture of the macrocanalicular system among bones from different species, different bones of the human body, and different parts of a single bone, utilizing this technique, would probably be most rewarding.

That the configuration of the macrocanalicular system of bone can be influenced by a disease process is illustrated in preparations from a case of Paget's disease (Fig. 7). In Paget's disease the ratio of bone destruction and bone synthesis is disturbed. The natural phenomenon of remodeling appears to occur at a more rapid rate than normal in this condition. This results in the typical histological picture of mosaic pattern seen on cross section, and increased volume of the marrow-vascular space. These changes were evidenced in our preparations, as indicated (1) by a widening of the general diameter of the Haversian systems and anastomosing Volkmann canals, and (2) by blunting or rounding off of junction of anastomoses, as compared with the sharp, right-angle anastomoses found in normal bone. We feel, therefore, that this technique may also prove to be a useful method of further understanding pathological changes in the matrix of bone. The method herein described of demonstrating the structure of the macrocanalicular system in ossified tissue is a simple one and may be applied without undue expenditure of time or money in any moderately equipped laboratory.

Summary

A technique has been developed in which a plastic model of the marrow-vascular space of bone can be prepared. It is felt that this technique may be utilized as an approach to the basic study of the morphology of normal and diseased bone. Examples of these preparations are illustrated in this report.

We wish to express our appreciation to C. Andrew L. Bassett, M.D. (Orthopedic Research Laboratory) and to Emanuel B. Kaplan, M.D. (Department of Anatomy), Columbia University College of Physicians and Surgeons, for their valuable suggestions and encouragement.

Orthopedic Research Laboratory, Columbia University College of Physicians and Surgeons.

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Autoradiographic Localization of Thyroid Hormones in the Organs and Tissues of the Rat

RENATO BASERGA, M.D., Chicago

While the mechanisms of iodine uptake and hormone formation in the thyroid have been investigated in great detail,[1] comparatively little information is available on the distribution of thyroid hormones in the organs and tissues of the body. Although Abelin and Scheinfinkel[2] had shown, as far back as 1925, that thyroxine concentrates in the liver, it was only in 1949 that a more systematic investigation of the peripheral distribution of thyroid hormones was conducted by Gross.[3] Biochemical and autoradiographic studies of the distribution of thyroxine or triiodothyronine, labeled with I^{131}, were reported by Gross and Leblond[4,5] and, more recently, by Ford, Corey, and Gross.[6]

Gross and Pitt-Rivers,[7] however, pointed out that the physiological metabolism of thyroxine must be distinguished from the metabolism of abnormal amounts of thyroxine introduced into the body, and Myant and Pochin[8] found that the endogenous hormone is more stable in the body and less diffusible than exogenous thyroxine. Endogenously labeled thyroid hormone may be obtained if a sufficient time is allowed to elapse after the injection of radioactive iodine. In a few days virtually all the inorganic iodine administered, and not collected by thyroid, has been eliminated, and essentially all of the I^{131} present in the body represents labeled thyroid hormones or radioactive metabolites of it.[9] As a knowledge of the target organs of the thyroid hormones might conceivably be of some interest, we attempted, in the present experiment, to investigate the peripheral distribution of endogenous thyroid hormones by autoradiography. To increase the yield and the accuracy of positive autoradiographs, two other procedures were used: the administration of thyroid-stimulating hormone (TSH), which accelerates the release of hormones from the thyroid gland, with a peak after two to three hours,[10,11] and the stripping-film technique,[12] which gives a more detailed autoradiographic localization.

Submitted for publication March 20, 1959.

Department of Pathology, Northwestern University Medical School.

Methods and Materials

Seventeen white rats, male and female, weighing from 260 to 315 gm., were injected intraperitoneally with iodine-131 (I^{131}) in the form of sterile, carrier-free sodium iodide in saline.* Two different dose levels were used: One group, of 4 rats, received 25μc. of I^{131} and were killed two days later, while a second group, of 13 rats, received 250μc. of I^{131} and were killed three days later. With the exception of four rats (two of each group), all the others were injected subcutaneously, six hours before death, with 10 U. S. P. units of TSH (Thytropar†).

Sections were taken from the following organs: thyroid and neck organs, heart, lung, small and large intestines, liver, pancreas, kidney, spleen, adrenal gland, thymus, salivary glands, skeletal muscles, testis, epididymis, ovary, uterus and oviduct, urinary bladder, lymph nodes, and gallbladder. The tissues were fixed in absolute alcohol and embedded in paraffin, following routine histological procedures. In addition, the tibias and femurs of four animals, all of which had received 250μc. of I^{131} and 10 U. S. P. units of TSH were fixed in acetone and processed according to the method described by Woodruff and Norris.[13]

Autoradiographs of the deparaffinized sections were taken both by the contact and by the stripping-film method. For the contact technique‡ Kodak Autoradiographic Plates, 1×3 in., were

* Abbott Laboratories, North Chicago, Ill.

† The Armour Laboratories, Chicago.

‡ Eastman Kodak Company, Rochester, N.Y.

Fig. 1.—Stripping-film autoradiograph of thyroid and trachea of rat, three days after the administration of I^{131}. The blackened portions represent the lateral lobes of the thyroid. × 5.8.

used. The exposure time was 24-30 hours for the thyroid sections and 12 to 26 days for all other sections. Only those autoradiographs that were visible to the naked eye were considered positive. For the faintest autoradiograph, this corresponded to a grain count of about twice the background. For the stripping-film technique,[14] a nuclear-track emulsion § (Ilford G.5) was used.

The exposure time varied from 24 to 30 hours for the thyroid sections and from 20 to 33 days for all other sections. After development and fixation, the sections were stained with Giemsa stain, according to the procedure of Gude, Upton, and Odell,[15] air-dried, and mounted in balsam.

Results

In the four animals injected with 25μc. of I^{131}, only the thyroids gave positive autoradiographs, while all the other organs examined were autoradiographically negative. In the 13 rats receiving 250μc. of I^{131}, the thyroids gave strongly positive images (Fig. 1), and in the other organs the results were as follows:

§ Ilford, England.

Heart.—The contact method gave faint images in four instances, and was negative in the other nine instances. With the stripping technique, the number of grains above the sections was not appreciably greater than in the background.

Lungs.—The contact technique gave faint images in four cases (Fig. 2); all other instances and all strippings were negative.

Small Intestine.—Good autoradiographs were found in two contact plates, and faint images were found in another four cases. In the strippings, an increase of the number of grains above that of the background was found once in the lumen and twice above the bottom of the glands.

Large Intestine.—By the contact method, images varying from good to very strong (Fig. 3) were obtained in 10 instances. In one case there was a faint image, and two cases were negative. The strippings showed that most of the radioactivity was limited to the contents of the lumen (Fig. 4). Above the mucosa, however, the number of grains was greater than that in the background, while the muscular coats did not reveal any significant increase. In one rat, a chronic inflammatory granuloma, 5 mm. in size, was found to involve the muscular coats and the serosa of a segment of large bowel. Histologically, it consisted mostly of mononuclear cells, with a few polymorphonuclear cells. A radioactive spot, considerably stronger than the radioactivity of the wall of the bowel, was found to correspond to the site of this inflammatory process (Fig. 5).

Liver.—Good to very strong autoradiographs were obtained in eight contact plates (Fig. 6), and faint images in another

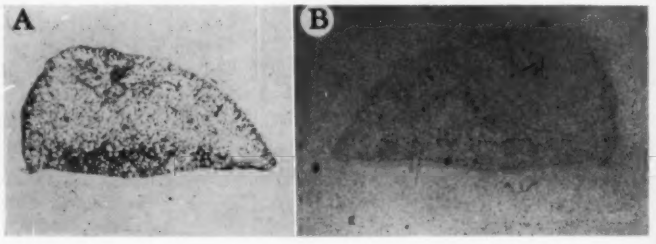


Fig. 2.—Photomicrograph (*A*) and contact autoradiograph (*B*) of lung of rat, three days after the administration of I^{131}. The slight blackening of the autoradiograph, which is the mirror image of the tissue section, is indicative of a moderate radioactivity. × 5.8.

Fig. 3.—Photomicrograph (*A*) and contact autoradiograph (*B*) of adrenal gland, large intestine and heart of rat, treated as above (Fig. 2). Note the blackening of the autoradiograph corresponding to the large intestine in the middle and the absence of radioactivity in the adrenal gland and heart. Reduced to 60% of mag. × 5.8.

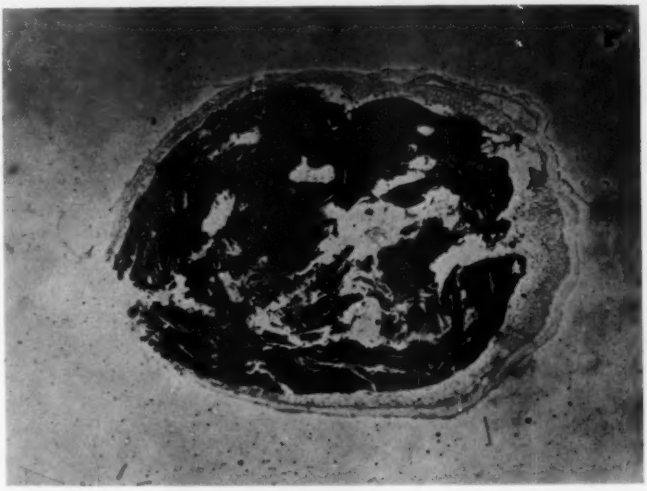


Fig. 4.—Stripping-film autoradiograph of large intestine of rat, treated as above (Fig. 2). The radioactivity is confined to the contents of the lumen. Reduced to 96% of mag. × 15.

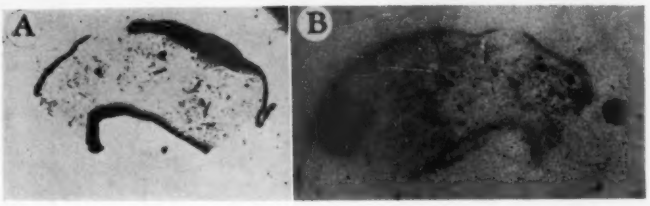


Fig. 5.—Photomicrograph (*A*) and contact autoradiograph (*B*) of large intestine of rat, treated as above (Fig. 2). There is blackening of the autoradiograph corresponding to the thickening of the wall, which is the site of an inflammatory process. Reduced about 88% of mag. × 5.8.

three; and two cases were negative. In all the cases that were positive with the contact method, the strippings showed a marked and diffuse radioactivity all over the sections (Fig. 7), but no cellular localization was possible.

Kidneys.—By the contact method, good images were obtained in seven instances (Fig. 8), and faint images, in the other six cases. These findings were confirmed in the strippings, where occasionally diffuse radioactivity was found concentrated above the tubular epithelium of the outer portion of the cortical zone. Deeper in the cortex, the tubular epithelium showed less radioactivity but contained deposits of a golden-brown pigment.

Salivary Glands.—Faint images were found in three contact plates, but in all these instances the strippings revealed that

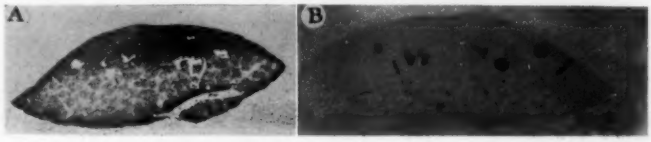


Fig. 6—Photomicrograph (*A*) and contact autoradiograph (*B*) of liver of rat, treated as above (Fig. 2). Reduced to 63% of mag. × 5.8.

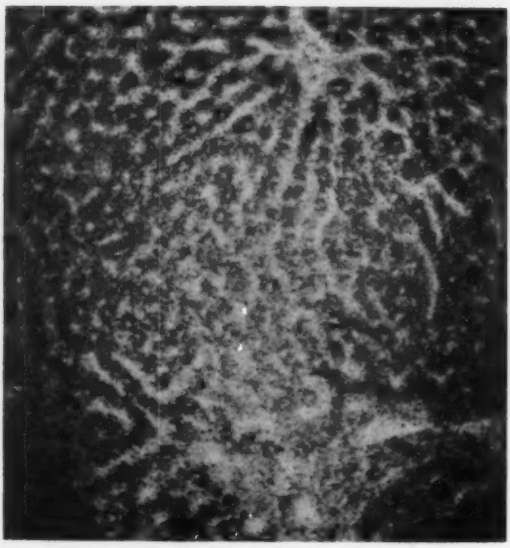


Fig. 7.—Stripping-film autoradiograph of liver of rat, treated as above (Fig. 2). Grains in focus; tissue slightly out of focus. × 160.

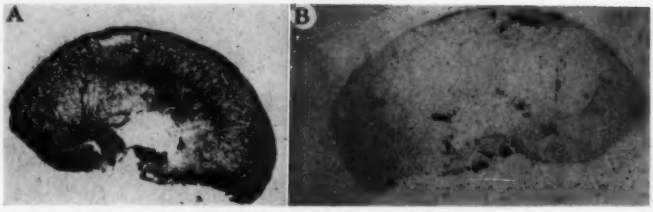


Fig. 8.—Photomicrograph (*A*) and contact autoradiograph (*B*) of kidney of rat, treated as above (Fig. 2). Reduced to ⅔ of mag. × 5.8.

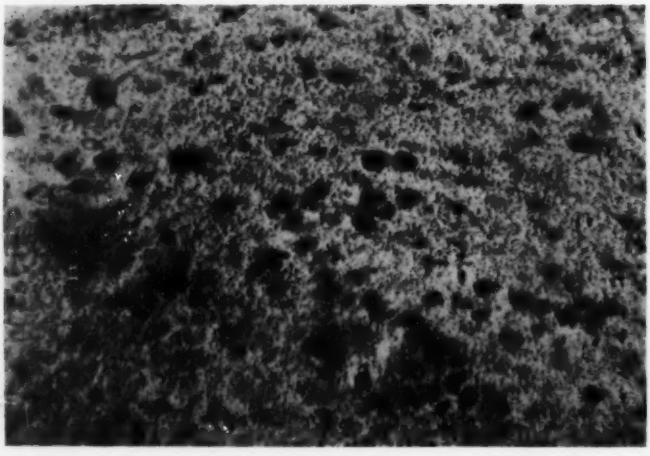


Fig. 9.—Stripping-film autoradiograph of uterus of rat, treated as above (Fig. 2). Note the concentration of grains above cells, which were found to be eosinophils. Reduced to 96% of mag. × 475.

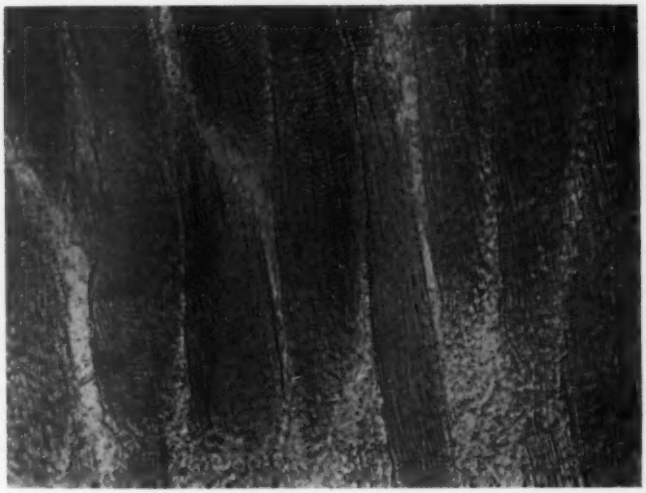
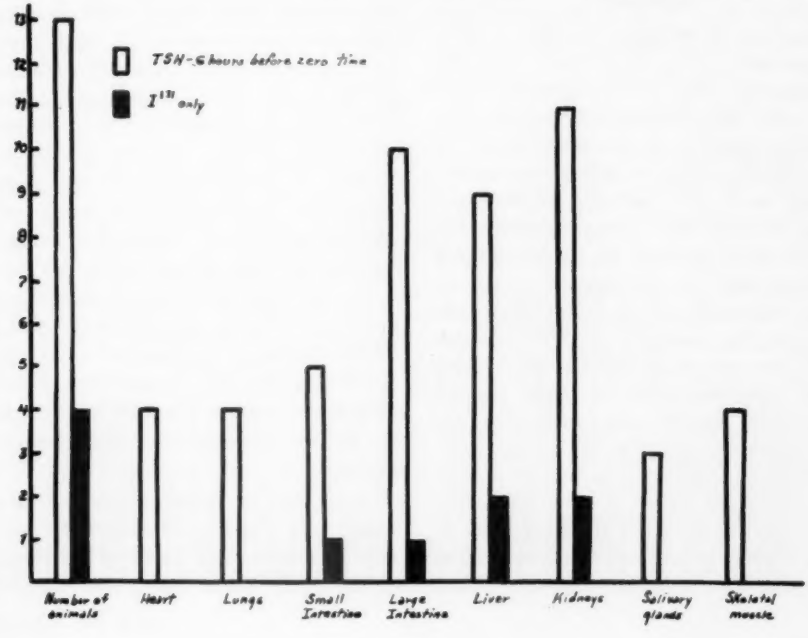


Fig. 10.—Stripping-film autoradiograph of skeletal muscle tissue of rat, as above (Fig. 2). Note the diffuse radioactivity. Reduced to 96% of mag. × 520.

Incidence of Positive Autoradiographs in the Tissues of Rats Seventy-Two Hours After Injection of I^{131}

Material Injected	No. Rats	No. of Positive Autoradiographs in							
		Heart	Lungs	Small Intestine	Large Intestine	Liver	Kidneys	Salivary Glands	Skeletal Muscle
I^{131} 250μc.	2	0	0	1	1	2	2	0	0
I^{131} 25μc.	2	0	0	0	0	0	0	0	0
I^{131} 250μc.+TSH	11	4	4	5	10	9	11	3	4
I^{131} 25μc.+TSH	2	0	0	0	0	0	0	0	0

Each column of the Table is illustrated by the corresponding column in the chart.



the radioactivity had leached outside the sections, indicating the presence of water-soluble I^{131}.

Uterus.—Contact plates of the uterus were negative. In two rats, however, the uterine mucosa showed focal accumulations of eosinophils, especially around the blood vessels. In these two animals the strippings revealed definite spots of radioactivity corresponding to the aggregating eosinophils (Fig. 9).

Skeletal Muscle.—Faint images were obtained in four contact plates, and in three of the corresponding strippings spots of radioactivity were found in the fibers (Fig. 10).

Other Organs.—The adrenal glands, thymus, pancreas, spleen, testis and epididymis, oviduct and ovaries, urinary bladder, lymph nodes, gallbladder, bones, tracheal cartilage, and esophagus were constantly negative with both methods.

The results are summarized in the Table. It should also be added that the more strongly positive autoradiographs were found always in TSH-treated rats.

Comment

Nature of the Radioactivity Revealed by the Autoradiographic Technique.—As mentioned before, the present investigation assumes that the radioactivity revealed by autoradiography represents the distribution of thyroid hormones or radioactive metabolites of it. It may be objected that one cannot be sure that the autoradiographs thus obtained are due to labeled thyroid hormones, and not to remnants of the injected radioiodide or to thyroglobulin released by the damaged thyroid. In this respect, the use of exogenous and labeled thyroid hormones offers a higher degree of certainty, but the assumption that our autoradiographs are due to the presence of endogenous thyroid hormones is corroborated by three facts: 1. Our rats were killed after 72 hours, a sufficient interval for the thyroid to take up radioactive iodine and release it as hormone. The radioiodine which is not so taken up is mostly eliminated within 24 hours in the urine and in the feces.[4] 2. The routine histological procedures that have been used are known to eliminate from the tissues whatever water-soluble inorganic iodine is present.[16] By using a similar routine procedure, Ford, Corey, and Gross[6] obtained negative autoradiographs in a guinea pig injected with I^{131} and killed two hours after the injection. If the autoradiographs are due to organic iodine, then most of the radioactivity is due to thyroid hormones, as these account for almost all the organic iodine normally present in the body.[17] It is true that after large doses of radioiodine, a butanol-insoluble fraction of protein-bound iodine, probably thyroglobulin, appears in the plasma[18]; but, at least in the rat, doses of the order of 800μc. to 1,000μc. are required for such an effect, whereas with a dose of 200μc. 97% of the protein-bound iodine of the plasma can still be identified as thyroxine.[19] 3. The stripping technique, which requires "subbing" of the section in distilled water, adds a further cause for the removal of water-soluble iodine. We found leaching of the radioactivity outside the sections only in the salivary gland, where inorganic iodine is excreted in copious amounts in the saliva.[20] However, Doniach and Logothetopoulos[21] found, after injection of radioiodine, that autoradiographs of the salivary glands were negative if processed as in the present experiment, and that positive images were obtained only with frozen-dried tissues. Perhaps the water-soluble iodine we found in the salivary glands is a product of the deiodination of the thyroid hormones.[7] In all other instances the procedures used seem to indicate that the radioactivity of our sections was protein bound,[21] and therefore due for the most part to labeled thyroid hormones.

Distribution of Thyroid Hormones in the Organs and Tissues.—Our results are in good agreement with those of biochemical studies,[4,5,22,23] which show that thyroxine

and triiodothyronine concentrate mainly in the liver, the contents of the large intestine, and kidneys. Since our rats were killed six hours after injection of TSH, the high radioactivity in the contents of the large bowel corresponds quite closely to the findings of Johnson and Albert,[24] who found that the radioactivity in the contents of the large bowel reached a peak six hours after injection of 3.8μg. of thyroxine. On the other hand, our results are at variance with the findings of Ford, Corey, and Gross,[6] who, however, used doses corresponding to 38μg. of thyroxine and 18μg. of triiodothyronine. These authors found that most organs gave positive autoradiographs. Perhaps smaller doses in the physiological range, that is, between 0.007μg. and 0.07μg.,[4] may have given different results, since the biochemical determinations of Gross and Leblond showed that the distribution of endogenously labeled hormone parallels that of tracer doses of thyroxine. Our results are also at variance with those of Godwin et al.,[25] who were unable to obtain positive autoradiographs from the extrathyroidal tissues of nine patients injected with radioiodine. Seven of their patients, however, died from 6 to 35 days after receiving the radioisotope, and of the other two, one, who died after eight hours, had some detectable radioactivity in the salivary gland and the spleen, and the second, although she died after two days, had received only 10 mc.

An additional finding in our material is the presence of radioactivity in an inflammatory process of the wall of the large bowel and in focal aggregates of eosinophils in the uterine mucosa. Heat, of course, is one of the cardinal signs of inflammation, and Gessler[26] attributed the increased heat of inflamed tissues to a higher oxygen consumption. Florey,[27] however, states that this increased metabolism is not sufficient to play any significant part in the rise of temperature. Perhaps our finding of a significant concentration of thyroid hormones in inflamed tissues may account, at least in part, for the increased heat production. It is even more difficult to explain the presence of radioactivity in the eosinophils, although these cells are known to be related to protein catabolism.[28] The remote possibility that the eosinophils might be responsible for the breakdown of thyroid hormones is suggested by the findings of Berg,[29] who showed that the eosinophilic granulations contain relatively large amounts of tyrosine, and by those of Aschkenasy,[30] who found eosinophilia in Graves' disease and in rats receiving thyroxine, and eosinopenia in myxedema and in thyroidectomized rats.

Detailed Localization of Thyroid Hormones.—I^{131} decays by emission of both beta particles and gamma photons. The beta particles, 87.2% of which have an energy of 0.608 mev, are mainly responsible for the autoradiographic image. The highly penetrating gamma rays, being uncharged, do not produce a direct latent image in the photographic emulsion: The ionizing action of gamma photons is due to photoelectric effect whereby electrons are ejected from atoms and molecules encountered by the radiation, and to the recoil electrons produced by Compton scattering. For these reasons, gamma rays decrease the resolution of autoradiographs and give poor results. In I^{131}, however, gamma photons constitute only 8% of the whole radioactivity[31]; and, as the dose of I^{131} in extrathyroidal tissues is of the order of about one-hundredth the injected dose,[32] it is reasonable to assume that the effect of gamma photons on the autoradiographs produced by small doses of labeled thyroid hormones is minimal. Furthermore, as good autoradiographs have been obtained with beta emitters of higher energy than I^{131}, there is no theoretical reason why cellular localization of I^{131} may not be achieved. Most of our strippings, however, show a diffuse radioactivity of the whole section, and this is especially true of the liver. On the other hand, cellular localization has been obtained in the focal aggregates of eosinophils in the uterine mucosa,

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and this seems to indicate that the diffuse radioactivity of the liver and other organs is the expression of a diffuse distribution of the thyroid hormones in the tissues, without preferential localization in one group of cells.

Summary

In conclusion, the present experiment, while confirming previous biochemical findings on the distribution and fate of thyroid hormones in the body, indicates that by the use of proper methods the peripheral distribution of endogenous thyroid hormone or its metabolites may be determined by autoradiography and the cellular localization ascertained. The number of positive autoradiographs is increased by the administration of thyroid-stimulating hormone (TSH) a few hours before death. It is conceivable that preparation of the animals with a low-iodine diet and treatment with thiouracil may increase further the amount of labeled thyroid hormones present in the tissues.

Thanks are due to Miss M. Krivich for technical assistance. The photographs were taken by Miss V. Morrison and Mr. A. Tracy.

Northwestern University Medical School, 303 E. Chicago Ave. (11).

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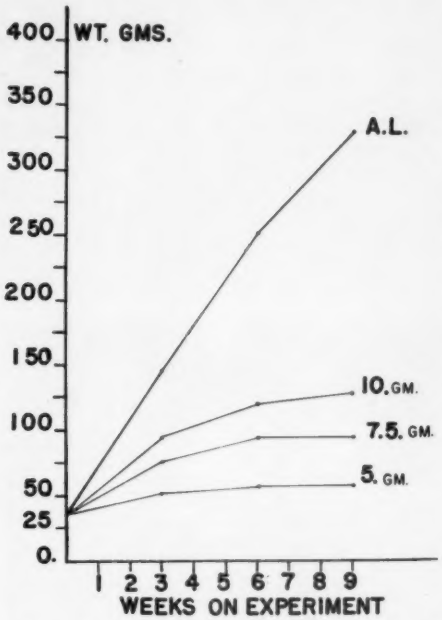

Fig. 1.—Comparative growth increment of ad libitum-fed rats versus that for the rats receiving 10, 7.5, and 5 gm. per day of the natural stock ration.

is directly proportional to the energy intake. The food intake of the ad libitum-fed animals (Group I) averaged 12 gm. per day by the end of the first week of the experiment. Their food consumption continued to increase with time, reaching a plateau level of approximately 24-26 gm. per day by the end of the third week of the experiment. Thus, from that time on, experimental Groups II, III, and IV received approximately 40%, 30%, and 20%, respectively, of the caloric intake of Group I (Fig. 2).

Three Weeks: The average widths of the epiphyseal cartilages in all experimental groups at the end of the three-week period on the experiment (42 days of age) are seen in Figure 3. There is a progressive decrease in the widths, which is proportional to the degree of caloric restriction. Figure 4 is a section of the epiphyseal cartilage from an ad libitum-fed animal. The average width measured 365μ. The four typical zones are present: 1. The resting zone, in which immature chondrocytes are scattered sparingly in the intercellular ground substance. 2. Zone of proliferating cells, 20-30 layers thick. These cells are flattened in shape and are arranged in parallel columns. 3. The hypertrophied layer, consisting of large, maturing cells rich in glycogen and arranged also in parallel columns. These cells are three to five layers thick. 4. The zone of calcifying cells adjacent to the diaphysis. The trabeculae projecting into the diaphysis are long and slender.

Figure 5 represents a section of the epiphyseal cartilage from an animal main-

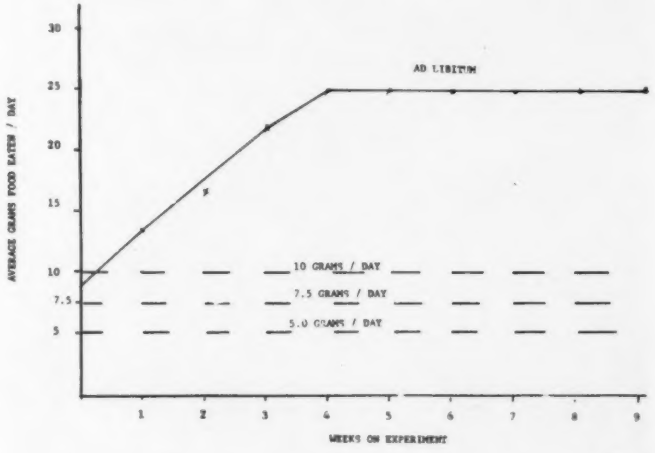

Fig. 2.—Food consumption of the ad libitum-fed animals versus that of the restricted groups.

Effects of Caloric Restriction on the Bones and Periodontium in Rats

L. A. BAVETTA, Ph.D.; S. BERNICK, Ph.D., and B. H. ERSHOFF, Ph.D., Los Angeles

Caloric insufficiency is at present one of the major causes of malnutrition in many parts of the world. Surveys of the Food and Agriculture Organization (FAO)[1] inform us that large areas of the world are more concerned with problems of caloric insufficiency than any other dietary constituent. The primary importance of food energy to the maintenance and growth of living systems has long been recognized. However, in recent years emphasis has shifted from caloric intake to vitamins, to the quality and relative amounts of protein, carbohydrate, and fat required, to balances among them, and, finally, to detailed considerations of balances among the proteins and their constituent amino acids.

Previous findings[2-4] indicate that caloric restriction per se may significantly affect both the growth and the histological appearance of skeletal tissues. However, the effects of caloric restriction on alveolar bone, a structure which is clinically known to exhibit pathological changes which may not always be mirrored by similar changes in the long bones, has not been investigated. Previous experiments from this laboratory[5,6] have demonstrated pathological effects in bone and periodontium as a result of tryptophan or lysine deficiencies. Inasmuch as food intake is generally reduced under such amino-acid-deficient diets, and inasmuch as such caloric reduction per se may result in negative nitrogen balance, the following experiment was initiated in an attempt to study the effects of graded levels of inanition on the bones and periodontium of rats.

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Departments of Biochemistry and Nutrition, University of Southern California School of Dentistry and School of Medicine.

Experimental Method

Seventy-two male rats of the Holtzman strain were selected at 21 to 23 days of age and an average body weight of 43 gm. (range, 38 to 48 gm.) for the present experiment. Animals were divided into four comparable groups, consisting of 18 rats each, and were treated as follows: Group I was fed ad libitum a natural food stock ration adequate for normal growth and reproduction.* Groups II, III, and IV were fed the above diet at levels of 10, 7.5, and 5 gm. per day, respectively. Rats were kept in individual metal cages with raised screen bottoms and were fed daily. Water was provided ad libitum. Food consumption was measured daily for all rats. Six rats in each group were killed after three, six, and nine weeks of feeding, and the tibias and jaws were removed for histological study.† These were fixed immediately in 80% alcoholic Bouin's solution. They were then decalcified, employing 10% nitric acid in 10% formalin solution, dehydrated, and infiltrated in the routine manner for nitrocellulose embedding. The sections were cut at 20μ-30μ and stained with hematoxylin and triosin.

Results

Growth and Microscopic Examination of Tibial Epiphyses.—The comparative growth increment is seen in Figure 1 and

* Purina Laboratory Chow, Ralston Purina Company, St. Louis.

† Similar experimental groups were also set up, employing a purified casein-containing diet similar to the vitamin A-supplemented basal ration employed by Bavetta et al.[7] Findings on the purified ration were similar to those obtained on the stock ration.

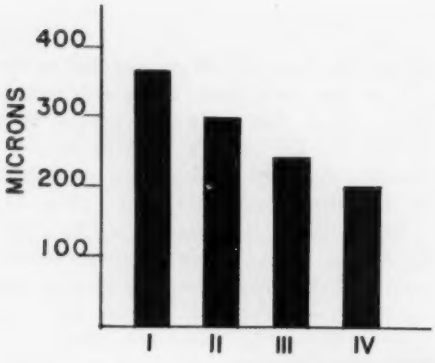

Fig. 3.—Average width of tibial epiphyseal cartilage plates of the ad libitum-fed group at the end of a three-week experimental period, compared with the width of the plates of animals on restricted intake.

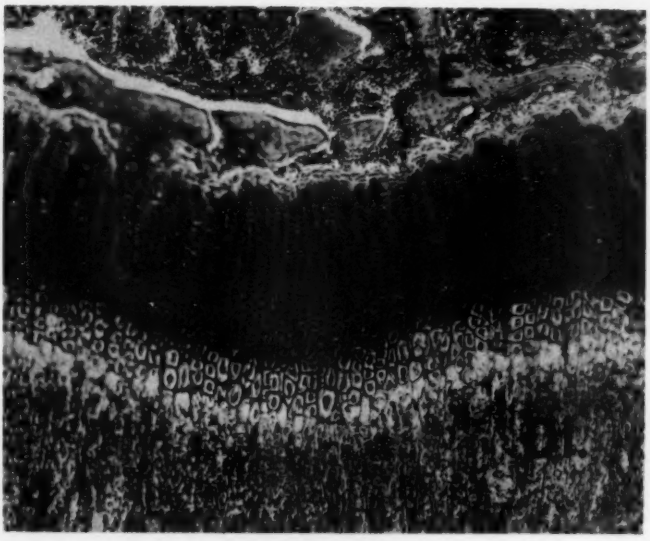

Fig. 4.—Proximal head of the tibia from an animal fed ad libitum for three weeks. Note the well-organized zones of the epiphyseal cartilage and the well-developed trabeculae in the diaphysis.

E., epiphysis; *E.P.*, epiphyseal plate; *DI.*, diaphysis. × 75.

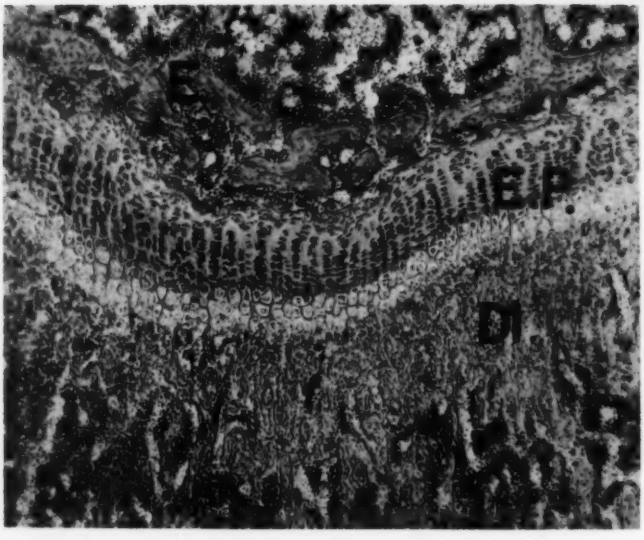

Fig. 5.—Proximal head of the tibia from an animal fed 5 gm. per day for three weeks. Although the epiphyseal cartilage is narrower and the cellular components smaller, endochondral ossification is still active, as seen by the presence of trabeculae extending into the diaphysis.

E., epiphysis; *E.P.*, epiphyseal plate; *DI.*, diaphysis; × 75.

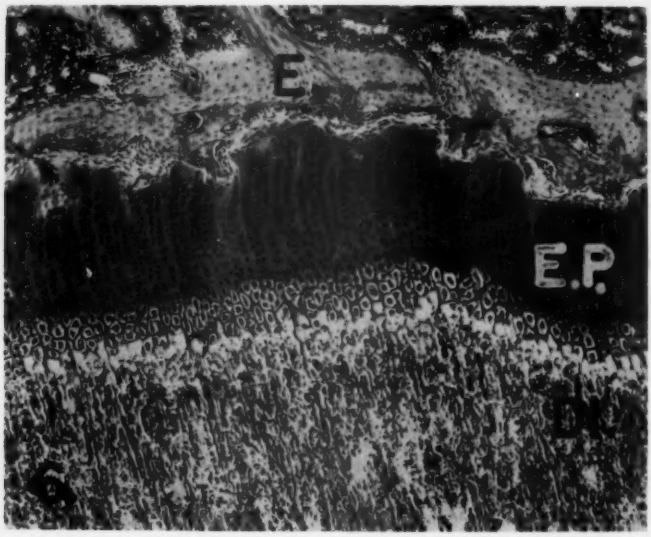

Fig. 6.—Proximal head of the tibia from an animal fed ad libitum for six weeks. The reduction in the width of the epiphyseal plate, and its cellular layers and components, is normal for animals of this age group. *E.*, epiphysis; *E.P.*, epiphyseal plate; *DI.*, diaphysis. × 75.

tained on 5 gm. of Purina Chow per day. The epiphyseal cartilage measured 200μ. Although the epiphyseal cartilage was narrower, all of the four characteristic zones are still evident, but there is a diminution in the size of the cellular components of all the layers. In addition, there is a marked decrease in the number of proliferating cells, proportional to the degree of caloric restriction. This zone is now 13-15 layers in thickness. At the same time there is a relative increase in intercellular ground substance. Endochondral ossification is still active, as seen by the presence of trabeculae extending into the diaphysis in all of the energy-restricted groups. Thus, except for the reduced epiphyseal plate widths, the morphological characteristics of the cartilaginous plates of the calorically restricted animals appear to be essentially normal,

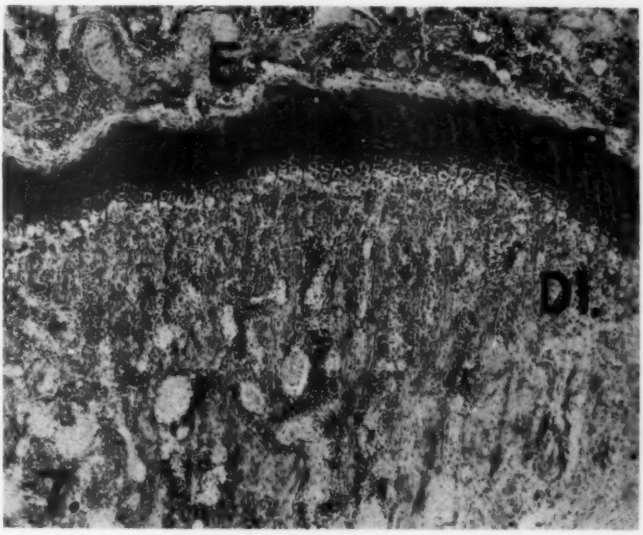

Fig. 7.—Proximal head of the tibia from an animal fed 5 gm. per day. The normal narrowing of the epiphyseal plate associated with aging is further accentuated. Observe the trabeculae projecting into the diaphysis. *E.*, epiphysis; *E.P.*, epiphyseal plate; *DI.*, diaphysis. × 75.

and growth is proceeding normally but at a reduced rate.

Six Weeks: A reduction in the width of the epiphyseal plates of all the animals, regardless of the amount of caloric intake, is observed during this experimental period. This decrease in width can be related to the physiological decreased growth rate characteristic of animals of this age group [7] and is a normal manifestation of the aging process. Figure 6 is a section of the epiphyseal plate from an animal fed ad libitum. As might be expected from an animal approaching maturity, there is a reduction in the width of the epiphyseal cartilage plate (275μ). This reduction is most conspicuous in the size and number of cells of the proliferating zone. At the same time, a normal increase in the amount of intercellular ground substance, also characteristic of "aging," was observed in both the control and the experimental animals.

Figure 7 exemplifies a section taken from an animal in Group IV at the end of six weeks. The epiphyseal plate measured 150μ. In spite of this narrowing, this decrease is related to a marked decrease in number of cells in the proliferating zone and may be interpreted as indicating a marked inhibition of protein synthesis. The trabeculae project into the diaphysis and are coarser than the controls.

Nine Weeks: At the 84th day there is a further narrowing of the epiphyseal plate in the control animals. As described above, this narrowing is a reflection of the decrease in cell numbers of the proliferating zone, and a further increase in intercellular matrix (Fig. 8).

The extension of the experiment to nine weeks does not markedly alter the epiphyseal plate of animals fed the calorically restricted diets. Figure 9 is a section from an animal fed 5 gm. per day. The width measures 135μ. The cellular components of all the layers are similar in size and number to that of Figure 4. However, the diaphyseal trabeculae are wider.

Alveolar Bone.—Three Weeks: The interradicular bone of the upper first molar from an animal fed ad libitum is seen in Figure 10. The bone is composed of two layers: the alveolar bone proper, consisting of bundle and lamellated bone, and the inner region, the spongiosa, containing anastomosing trabeculae that fuse with the alveolar bone proper. The marrow spaces contain myeloid elements.

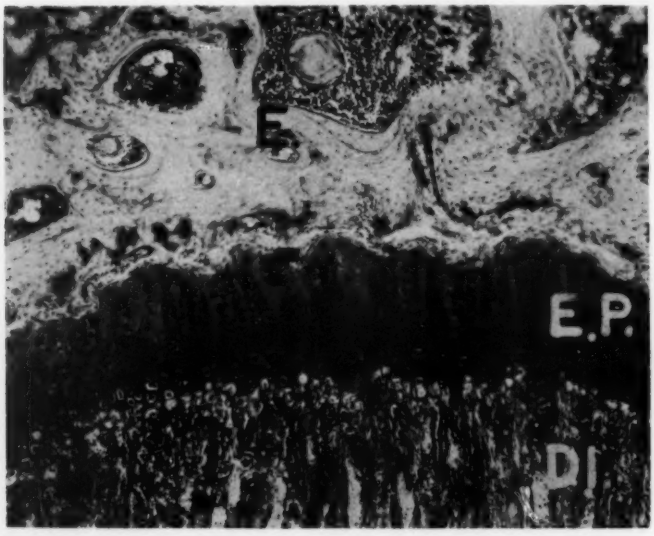

Fig. 8.—Proximal head of the tibia from an animal fed ad libitum for nine weeks. The narrowing is a reflection of the decrease in the cell numbers, especially in the proliferating zone.

E., epiphysis; *E.P.*, epiphyseal plate; *DI.*, diaphysis. ×75.

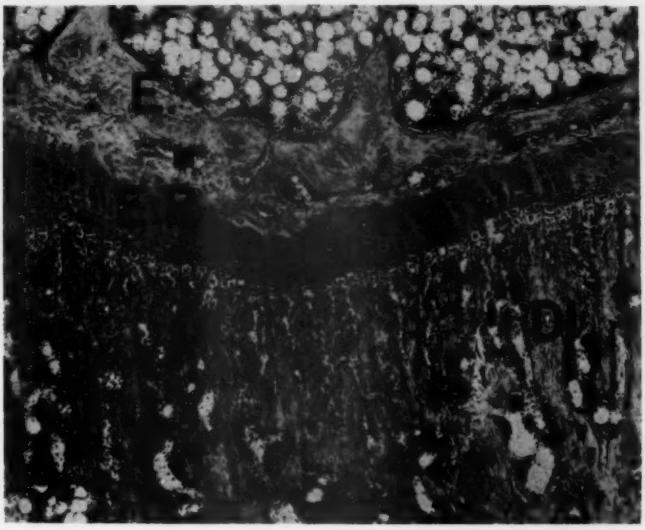

Fig. 9.—Proximal head of the tibia from an animal fed 5 gm. per day for nine weeks. Note that the extension of the restricted diet to a period of nine weeks does not alter the morphology of the epiphyseal plate.

E., epiphysis; *E.P.*, epiphyseal plate; *DI.*, diaphysis. × 75.

The animals subjected to various degrees of caloric restriction exhibit a slightly modified bone structure. The alveolar bone proper, however, remains unchanged in all cases. On the other hand, there is a thinning of the trabeculae of the spongiosa, with a corresponding enlargement of the marrow spaces. These changes may be seen in Figure 11, which is a section from an animal receiving 5 gm. per day for three weeks. However, it should be noted that these slender trabeculae are lined with osteoblasts. In this section, it is observed that the enlarged marrow spaces are filled with myeloid elements.

Six Weeks: At this time the alveolar bone of the animals in Group I exhibits a marked thickening of the spongiosal trabeculae (Fig. 12). These trabeculae have fused together at the crestal half of the bone, giving it a very dense appearance. Figure 13 is a similar section from a calorically restricted animal (Group IV). It can be easily observed that these animals also

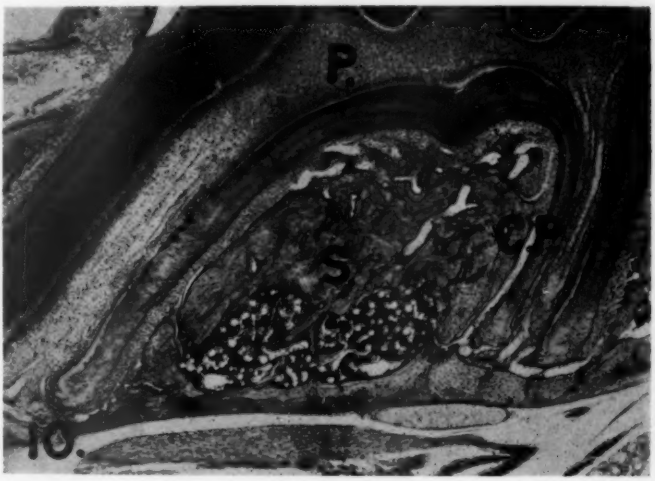

Fig. 10.—Interradicular bone of the upper first molar from an animal fed ad libitum for three weeks. Note the dense lamellar, anastomosing trabeculae of the spongiosa that fuse with the alveolar bone proper.

P., pulp; *C.P.*, alveolar bone proper (cribriform plate). Reduced to 88% of mag. × 35.

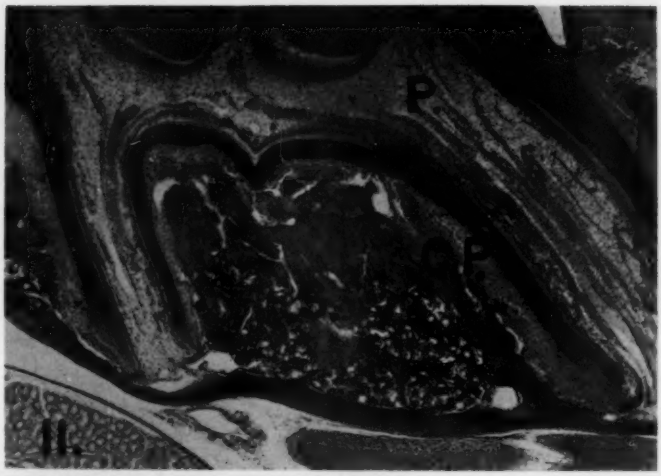

Fig. 11.—Interradicular bone of the upper first molar from an animal fed 5 gm. per day for three weeks. Note the alveolar bone proper remains unchanged, whereas the trabeculae of the spongiosa are thinner.

P., pulp; *S.*, spongiosa; *C.P.*, alveolar bone proper (cribriform plate). Reduced to 88% of mag. × 35.

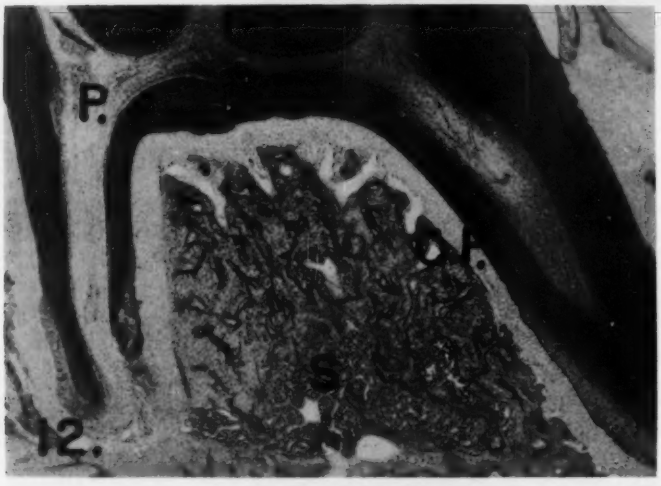

Fig. 12.—Interradicular bone of the upper first molar from an animal fed ad libitum for six weeks. Note that there has been a thickening of the spongiosal trabeculae, giving a very dense appearance.

P., pulp; *S.*, spongiosa; *C.P.*, alveolar bone proper (cribriform plate). Reduced to 88% of mag. × 35.

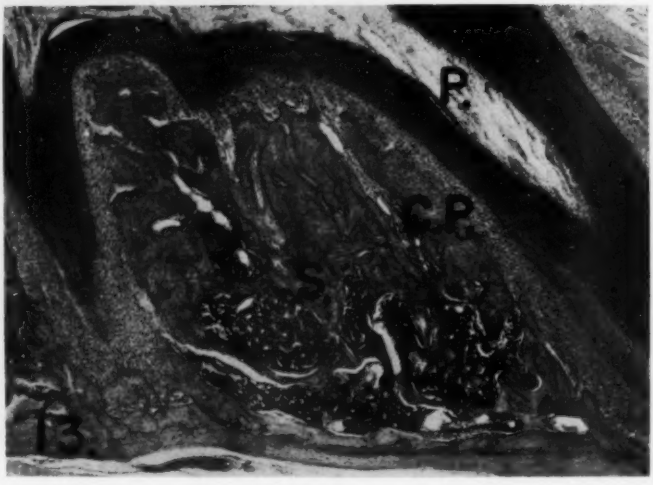

Fig. 13.—Interradicular bone of the upper first molar from an animal fed 5 gm. per day for six weeks. Note that the bone also exhibits a similar alveolar density.

P., pulp; *S.*, spongiosa; *C.P.*, alveolar bone proper (cribriform plate). Reduced to 88% of mag. × 35.

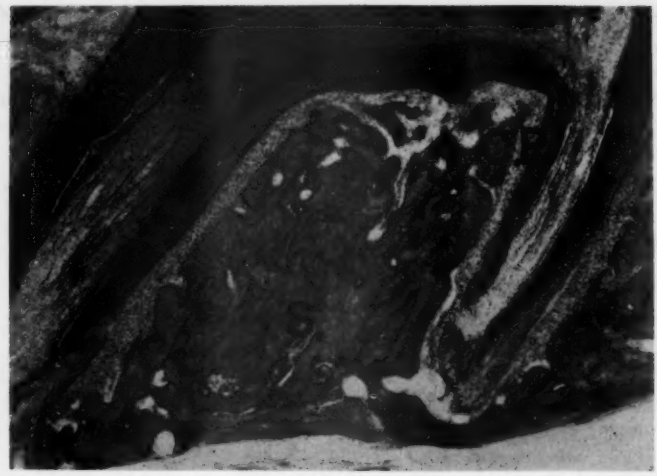

Fig. 14.—Interradicular bone of the upper first molar from an animal fed ad libitum for nine weeks. Note that at this age the alveolar bone is practically a solid mass.

P., pulp; *S.*, spongiosa; *C.P.*, alveolar bone proper (cribriform plate). Reduced to 88% of mag. × 35.

exhibit a similar alveolar bone density, and certainly do not demonstrate the marked osteoporosis previously observed in lysine and tryptophan deficiencies.

Nine Weeks: At this age the increased rate of bone deposition has led to the formation of practically a solid mass of alveolar bone in animals of Group I (Fig. 14). The alveolar bone in calorically restricted animals (Group IV), however, continues also to show an increase in the density of the bone, the crestal half of the spongiosa appearing as a solid mass (Fig. 15).

Comment

The occurrence of pathologic effects in animals fed diets deficient in an essential nutrient raises the question as to what extent such effects are specific for the particular factor under investigation or to what extent they may be due, at least in part, to an associated caloric restriction. Present findings indicate that under conditions of simple caloric restriction of a qualitatively adequate diet, cartilaginous proliferation is retarded but maintains its normal organization. The result is a sort of "miniature"

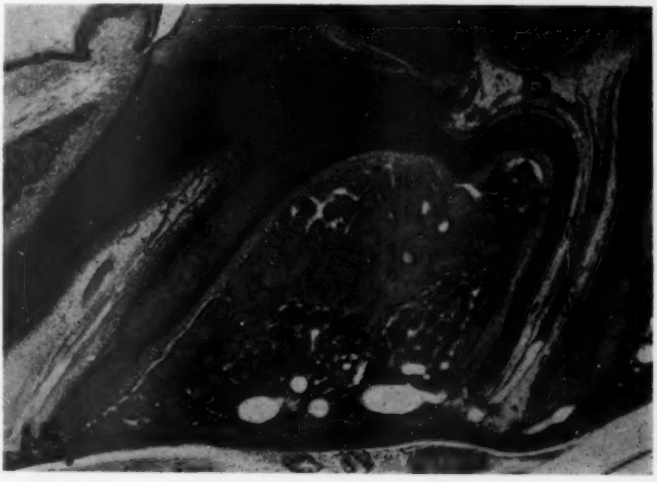

Fig. 15.—Interradicular bone of the upper first molar from an animal fed 5 gm. per day for nine weeks. Note that here also the bone shows an increase in the density of bone with the crestal half of the spongiosa appearing as a solid mass.

P., pulp; *S.*, spongiosa; *C.P.*, alveolar bone proper (cribriform plate). Reduced to 88% of mag. × 35.

of the normal. These findings are in contrast to the marked osteoporosis of both the alveolar and the long bones of rats fed diets deficient in tryptophan,[5] lysine,[6] and other essential nutrients. When immature rats were fed diets deficient in tryptophan or lysine,[5,6] loss of trabeculae was noted as early as three weeks after the start of feeding and became aggravated as the experimental period was prolonged. Under conditions of the present experiment, however, where immature rats were fed graded levels of a qualitatively adequate diet, no loss of trabeculation occurred in either the long bones or the alveolar bone. Rats fed diets deficient in tryptophan, lysine, and other essential nutrients lose weight continuously from the start of feeding. On the other hand rats fed a qualitatively adequate diet restricted in calories reach a plateau in body weight and maintain "caloric equilibrium," which is associated with the good bone of the latter group. Such equilibrium is apparently not possible in the absence of an essential nutrient.

Summary

Male rats were selected at weaning and were fed a natural food stock ration for periods of three, six, or nine weeks either ad libitum or at levels of 10, 7.5, or 5 gm. per day. No loss of trabeculation of either the long bones or alveolar bone occurred in any of the calorically restricted animals. Cartilaginous proliferation was retarded in the latter animals, but its normal organization was maintained.

Department of Biochemistry, University of Southern California School of Medicine (Dr. Ershoff).

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Pathologic Effects of Thermal Neutrons and the B^{10} (n, Alpha) Li^7 Reaction on Skin

S. W. LIPPINCOTT, M.D.; J. S. ROBERTSON, M.D., Ph.D.; V. P. BOND, M.D., Ph.D.; E. P. CRONKITE, M.D.; O. D. EASTERDAY, Ph.D., and L. E. FARR, M.D., Upton, N.Y.

Boron-10 (B^{10}) is a stable isotope which, upon capturing a thermal neutron, disintegrates into an energetic alpha particle and a lithium-7 (Li^7) particle. This reaction, symbolized by B^{10} (n,α) Li^7, is currently being used in a program of experimental therapy of brain neoplasms.[1] Stable boron administered intravenously becomes distributed at different rates among the various tissues, and the time of maximum concentration occurs sooner in brain neoplasms than in normal brain. It has been presumed, but not proved, that the optimal time for exposing the neoplasm thus loaded with boron to thermal neutrons is during the period when the neoplasm-brain boron concentration ratio is the highest. Occurrence of the B^{10} (n,α) Li^7 reaction within the neoplasm results in the liberation of short-range alpha and Li^7 particles capable of causing destruction in the neoplastic tissue.[2] In earlier investigations vexing skin reactions occurred at the site of irradiation, and a parallel animal study was initiated to study the nature of these lesions and to learn more about tissue response to radiation from thermal neutrons and energetic heavy particles. The pig was selected, since its skin is histologically more similar to that of man. In the present report the pathologic findings in the skin of swine exposed to thermal neutrons before and after the intravenous injection of boron are presented.

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Medical Department, Brookhaven National Laboratory.

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Materials and Methods

A total of 10 swine of the Duroc strain, of either sex and each weighing approximately 70 lb., were used in these studies. Two served as normal, unirradiated controls. Seven were anesthetized with pentobarbital (Nembutal), given intravenously, and were then exposed in the cheek and eye region or in the shoulder to thermal neutrons only for 30 minutes over a 10×10 cm. port in the Medical Facility of the Brookhaven Graphite Reactor. After this irradiation, the animals were given boron in the form of sodium tetraborate decahydrate (borax) intravenously, and were then exposed an additional 30 minutes in the region of the flank, over the same 10×10 cm. port, the starting exposure five minutes after boron injection. One animal did not receive the preliminary thermal-neutron irradiation, being exposed to thermal neutrons only after boron injection. All irradiated animals were exposed to approximately 5×10^{12} n/sq. cm., and boron doses ranged from 25 to 45 mg/kg. (Table). After exposure the animals were observed at frequent intervals and were killed at intervals up to 112 days after exposure, as indicated in the accompanying Table.

Boron was administered as borax in a glucose solution in the molar ratio of boron to glucose of 1:2. Thermal-neutron fluxes were measured by activation of gold foils placed at the proximal skin surface during exposure. Although the thermal-neutron flux delivered and the injected boron dose are known accurately, lack of detailed knowledge of the precise kinetics of boron distribution in tissues created considerable uncertainty in the translation to released dose, in rads. As a rough estimate, it can be calculated that 30 mg/kg. of boron and 5×10^{12} thermal neutrons/sq. cm. will result in an energy release of approximately 1,400 rads from the recoil alpha and lithium particles, and an additional 600 rads from other neutron reactions and from gamma rays.

Results

Gross Clinical Observations.—In the areas exposed only to thermal neutrons, minimal erythema was noted within 24

Experimental Irradiation Procedure in Ten Pigs

Pig	Exposure Area	Neutrons per Square Centimeter	Boron Dose, Mg/Kg.	Exposure Time, Min.	Day Killed After Exposure
S2156	Control	None	None		
S2343	Control	None	None		
S2155	Boron only	None	25		24
S1105 (7)	Cheek and eye	4.82×10^{12}		30	
		4.82×10^{12}	45	30	22
S1106 (8)	Cheek and eye	4.04×10^{12}		30	
		4.04×10^{12}	45	30	22
S1107 (9)	Cheek and eye	4.22×10^{12}		30	
		4.22×10^{12}	45	30	22
S1071	Head and cheek	6.3×10^{12}		30	
		6.3×10^{12}	40	30	33
S2157	Right shoulder	4.8×10^{12}		30	
	Right flank	4.8×10^{12}	25	30	15 (died)
S2158	Right shoulder	4.8×10^{12}		30	
	Right flank	4.8×10^{12}	25	30	25
S2345	Right shoulder	4.5×10^{12}		30	
	Right flank	4.5×10^{12}	25	30	112

hours. Recovery was rapid, and no further evidence of skin damage was observed grossly. In all areas exposed to both boron and thermal neutrons, marked edema and erythema developed within 24 hours. The edema and erythema subsided, to be followed within 10 days by severe epidermitis, which invariably progressed to ulceration. In those animals in which the eye was included in the field, marked inflammation of the lids, conjunctiva, and lens, leading to necrosis, was observed.

Pathology of Skin Lesions.—The gross and histopathological findings in skin following irradiation with electromagnetic waves, such as x-ray and gamma rays, have been well documented in the literature for several species. In contrast, little has been observed and recorded concerning alpha-particle radiation, particularly that produced experimentally in skin. Fortuitously, the microscopic features of normal skin from the pig are very similar to those of man; thus the pig makes a suitable species

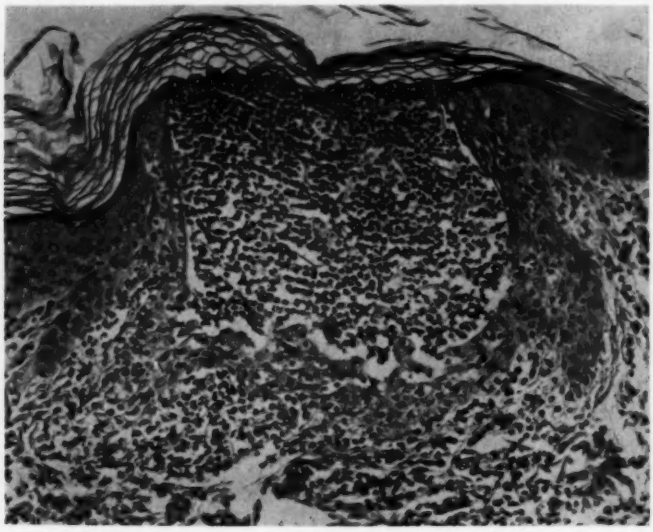


Fig. 1 (S2156).—Exudative epidermitis. Reduced to 80% of mag. ×512. Brookhaven National Laboratory, Photography Division, Neg. No. 3-92-58.

for investigation. A lesion which has been described in the literature as exudative epidermitis is shown in Figure 1, as it occurred in both control and experimental animals. In the original experimental work we did not know that this lesion occurred in pigs and were not looking for it as a minute gross lesion. We were, therefore, somewhat surprised to see it microscopically. Its importance in the experiments was that it be recognized and not be confused with what was occurring as a result of radiation. The lesion consists of a simple microscopic ulcerative defect confined to the epithelium. The circumscribed lesion consists of necrosis of the squamous epithelium, and the cellular constituents shown in the photomicrograph consist primarily of this debris, together with many leukocytes and occasional lymphocytes.

The appearance of degenerative changes in the epidermis occurred within 22 days and was characterized chiefly by complete loss of epithelial cells, with occasional remaining remnants of flattened squamous cells. In such areas irregular layers of hyalinized material persisted, sometimes with spaces containing bizarre cells. Figures 2 and 3 demonstrate these findings. Although the $n+B^{10}$ reaction was given to a 10 cm. square area on the skin surface, the radiation effect was not histologically en-

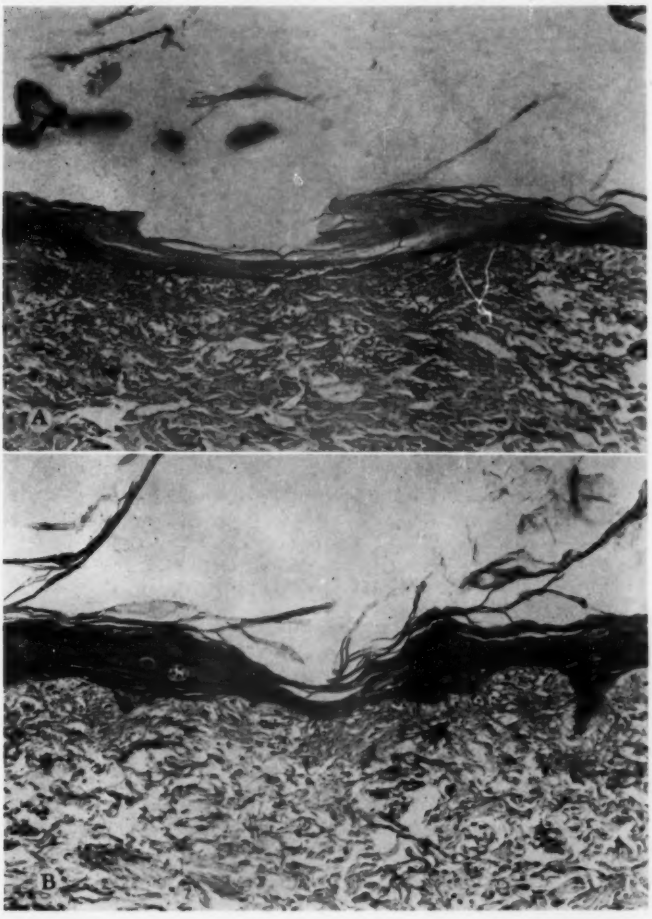


Fig. 2 (S1107).—*A* and *B*, atrophy of epithelium 22 days after exposure to 4.22×10^{13} n/sq. cm.$+B^{10}$. Reduced to 63% of mag. × 320. Brookhaven National Laboratory, Photography Division, Neg. Nos. 3-105-58 and 3-104-58.

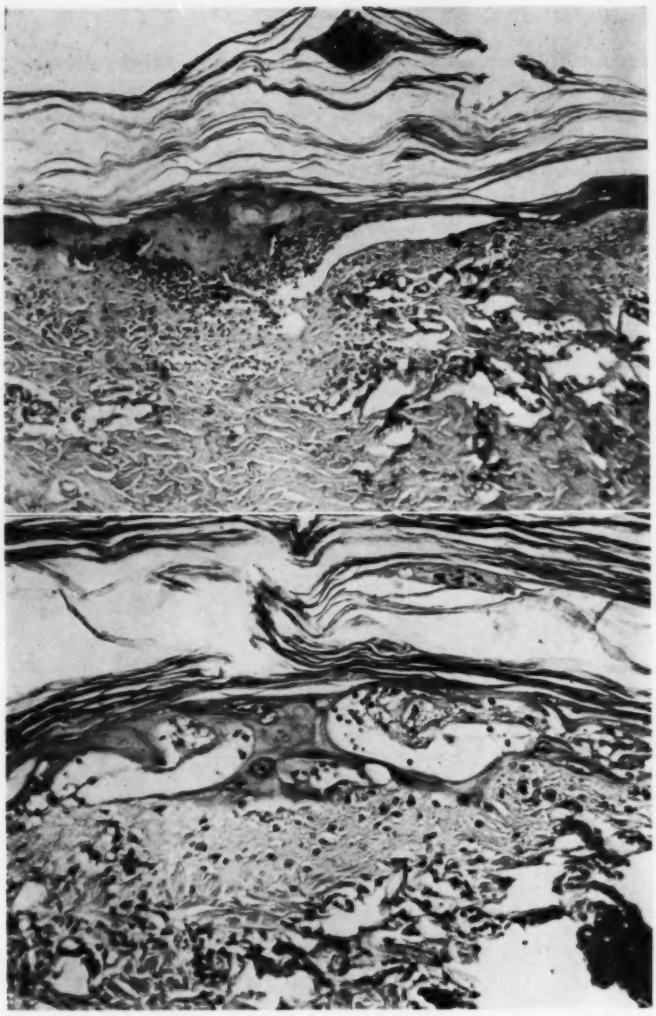
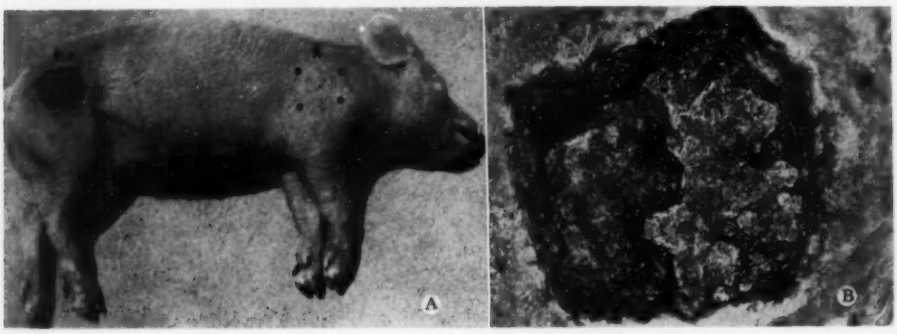


Fig. 3 (S2158).—Atrophy and degeneration of epithelium 25 days after exposure to 4.8×10^{13} n/sq. cm.+B^{10}. Reduced to 88% of mag. × 320. Brookhaven National Laboratory, Photography Division, Neg. Nos. C3-103-58 and C3-91-58.

Fig. 4 (S2345).—*A* and *B*, radiation ulcer at site of exposure to 4.5×10^{13} n/sq. cm.+B^{10}, 112 days after exposure to radiation. Brookhaven National Laboratory, Photography Division, Neg. No. 4-668-59.



tirely uniform, as shown by serial block sections taken throughout the 10 cm. square area and surrounding adjacent nonirradiated tissue. The atrophy is therefore irregular and nonuniform. Where such atrophic changes occurred in epithelium, there was essentially no underlying change in corium with reference to connective tissue and small vascular channels.

In Figure 4*A* no lesion is seen in the 4 in. (10 cm.) square area overlying the front right shoulder, where exposure to neutrons only was given. A comparable-sized area over the right flank shows a 4 in. square area of ulceration 112 days after exposure to 4.8×10^{12} neutrons/sq. cm. with a preceding injection of 25 mg. of B^{10} per kilogram of body weight and an exposure time of 30 minutes. The gross details of this ulcer are shown in Figure 4*B*. The margins of the ulcer are rolled, white, and firm. The necrotic base consists of laminated, tough

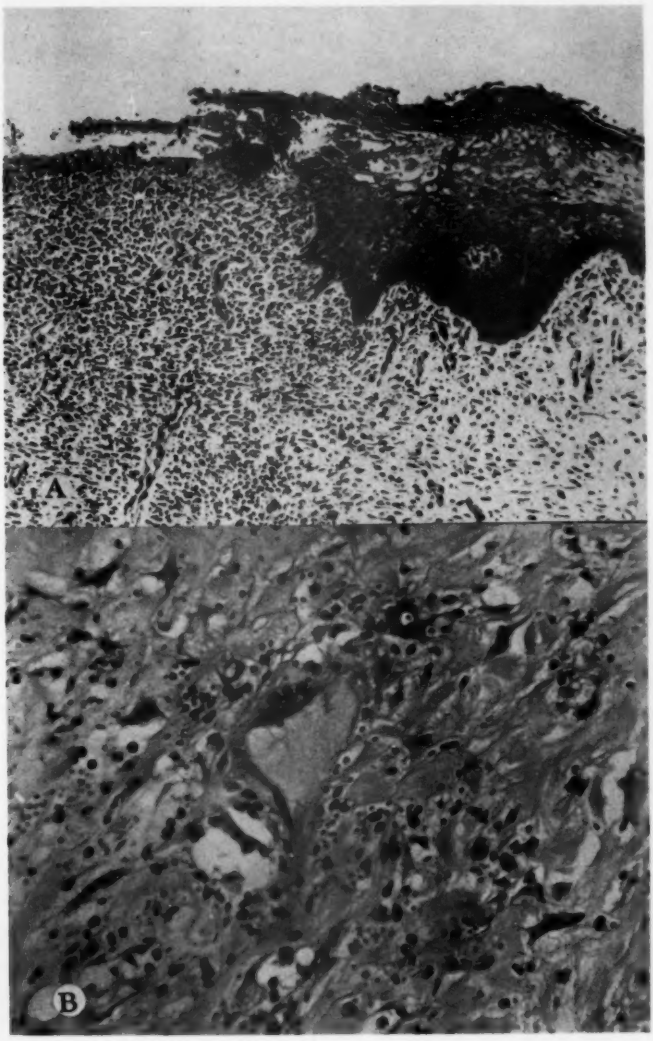


Fig. 5 (S2345). — *A*, margin of ulcer shown in Figure 4. Brookhaven National Laboratory, Photography Division, Neg. No. 3-106-58. *B*, base of ulcer in *A*, showing hypertrophied, bizarre endothelial cells in capillaries in granulation tissue. Reduced to 80% of mag. × 512. Brookhaven National Laboratory, Photography Division, Neg. No. 3-94-58.

tissue. The microscopic details of this ulcer are shown in Figure 5*A*. As would be expected from the gross appearance, this is an indolent ulcer with a firm granulation-tissue base. Although there is abundant connective tissue formation, there is no indication of a neoplastic process arising in the connective tissue. In many of the capillaries, hypertrophied and bizarre endothelial cells are seen (Fig. 5*B*). Only rarely in connective tissue cells of the stroma is an atypical cell seen. In the deeper portions of the base of the ulcer endothelial proliferation in arterioles (Fig. 6*A*) and small arteries (Fig. 6*B*) is seen to result in complete vascular obliteration.

An anesthetized pig, just prior to $n+B^{10}$ irradiation, is shown in Figure 7*A*, and below, in *B*, is shown an irregular ulcerative lesion of the eyelid, ear, and adjacent tissue from this animal 22 days after exposure to 4.2×10^{12} during 30 minutes after

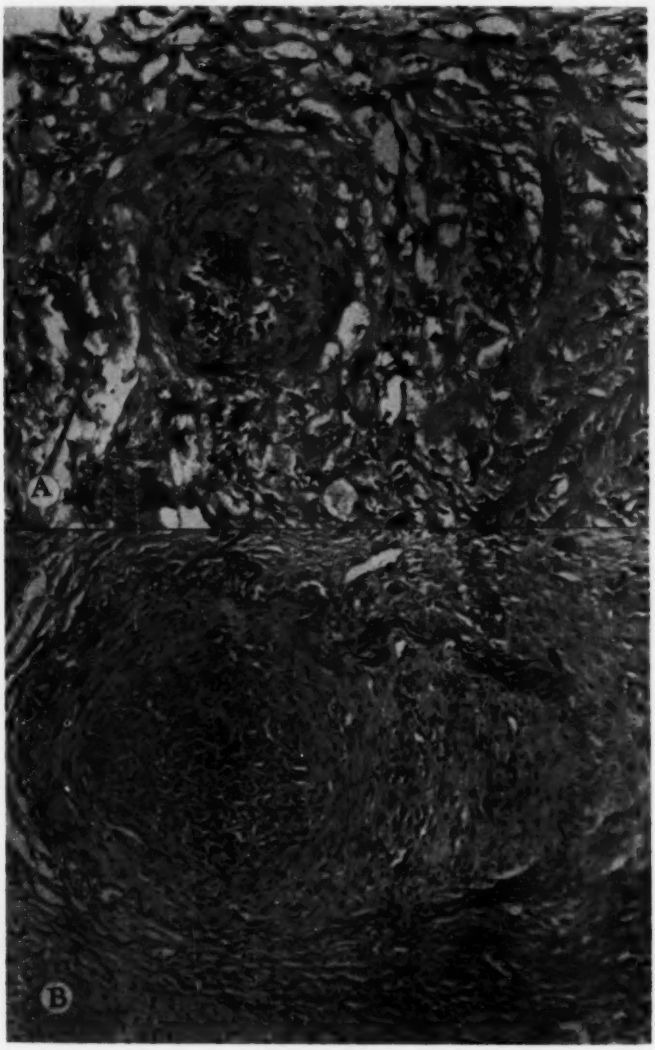


Fig. 6 (S2345). — *A* and *B*, endothelial and fibrous connective tissue proliferation obliterating arteriole (*A*) and small artery (*B*) from deep portion of base of ulcer (Fig. 4). Reduced to 80% of mag. × 512. Brookhaven National Laboratory, Photography Division, Neg. Nos. 3-88-58 and 3-86-58.

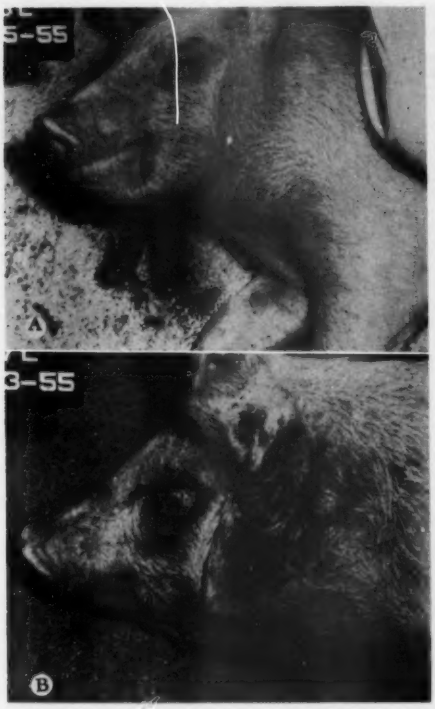


Fig. 7. (S1107 #9).—*A*, anesthetized pig before radiation exposure. Brookhaven National Laboratory, Photography Division, Neg. No. 8-353-5). *B*, same pig 22 days after exposure to 4.22×10^{13} n/sq. cm.+B^{10}, showing gross ulceration, in one area of which a squamous-cell carcinoma was found on microscopic examination. Brookhaven National Laboratory, Photography Division, Neg. No. 8-451-5.

administration intravenously of 45 mg. of B^{10} per kilogram of body weight. A totally unexpected finding from one of the blocks in the margin of this ulcerative defect in the ear is shown in Figure 8*A* and *B*. The lesion consists of a typical, small squamous-cell carcinoma. In consulting the literature, nothing was found indicating that squamous-cell carcinoma has been reported in the skin of the pig in early age periods. However, since there are not several pigs in this series with lesions of the ear following irradiation, a positive statement connot be made that this lesion is an induced squamous-cell carcinoma occurring 22 days after n+B^{10} irradiation. However, that this lesion might be spontaneous, occurring at this early age in a pig seems rather unlikely. The histological appearance of the actual neoplasm is shown in low- and high-power magnifications in Figure 8.

Pathology of Tongue Lesion.—In one pig whose tongue was exposed to n+B^{10} an oval ulcerated area occurred, and its appearance 16 days after irradiation is shown in Figure 9. The microscopic appearance of the lesion is seen in Figure 10 and consists of a recent ulcer in which can be distinguished very sharply the boundary of the intact squamous epithelium and the necrotic epithelium overlying muscle. Many leukocytes and fibrin mesh are present in the base of the lesion. The general outline of the lesion followed rather closely the predicted iso-dose exposure curve for thermal neutrons.

Comment

In this study it has been demonstrated that a single pig injected with boron only, and three pigs receiving thermal neutrons only over the right shoulder showed no histopathological lesions in the skin at the times of killing, ranging from 15 to 112 days. The microscopic appearance of the skin in these pigs was similar to that of the two control animals. In the three animals that received B^{10}, followed by thermal neutrons to the cheek and eye, and to the head and cheek in a fourth animal, the times of killing following radiation exposure varied from 22 to 33 days. The number of blocks of tissue taken from these areas of exposure was small, and it was because of the finding of one area of squamous-cell carcinoma that a second experiment was done. In the three pigs in this experiment the right shoulder was irradiated with neutrons only, and the right ham received neutron radiation following B^{10} injection. One animal died 15 days after treatment; a second was killed at 25 days, since the range in the first series had been 22 to 33 days, and the third pig was killed at 112 days. In each animal the area of irradiation of the skin was through a 10 cm. square portal; so at autopsy a 5-in.

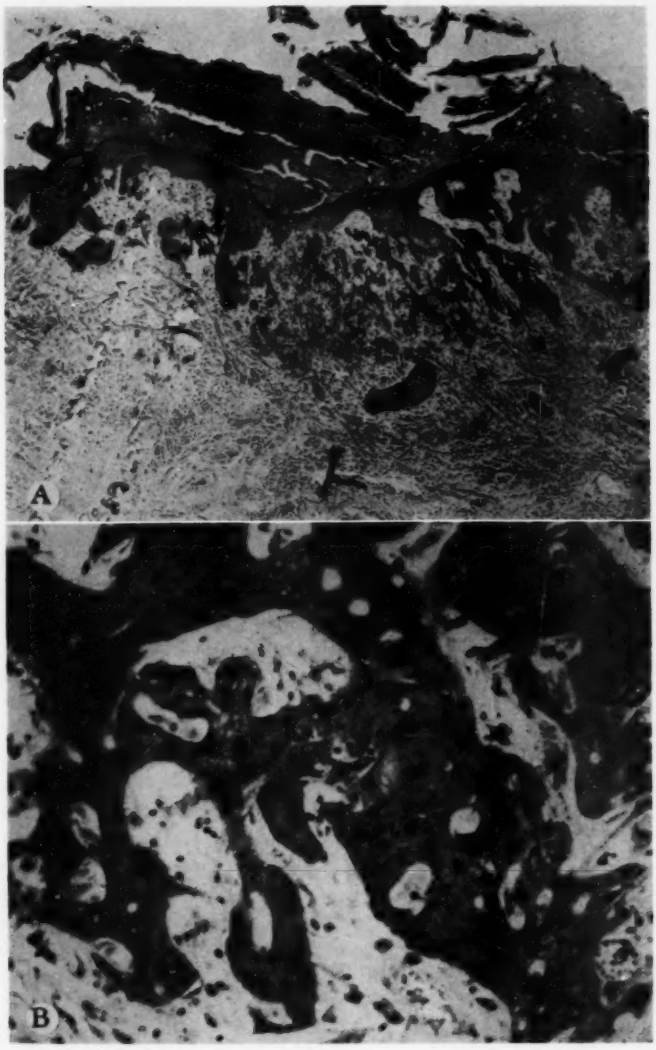


Fig. 8.—*A*, squamous-cell carcinoma of ear, region from ulcerated area shown in Figure 7*B*. Reduced to 80% of mag. ×80. Brookhaven National Laboratory, Photography Division, Neg. No. 5-472-58.

B, higher magnification of invasive neoplastic cells from selected area of *A*. Reduced to 80% of mag. ×510. Brookhaven National Laboratory, Photography Division, Neg. No. 5-470-58.

(12.7 cm.) square, including this 10 cm. square port, was removed in the region receiving neutrons only and in the region receiving neutrons following B^{10} injection. The entire area was sectioned into 1×½ in. blocks, from which microscopic sections were prepared. The objective was to find out whether the irradiation resulted in uniform histopathological findings in the exposed areas by the time the animals were killed. While this could be determined, this type of experiment gave no information as to effects at earlier time intervals, so that the exact pathogenesis of the lesion has not been established.

In general, if one had enough pigs, time, and facilities available, a series of experiments with animals killed at daily intervals would yield more extensive information than did our study. Such an experiment could help to resolve the important problem of whether primary degenerative effects oc-

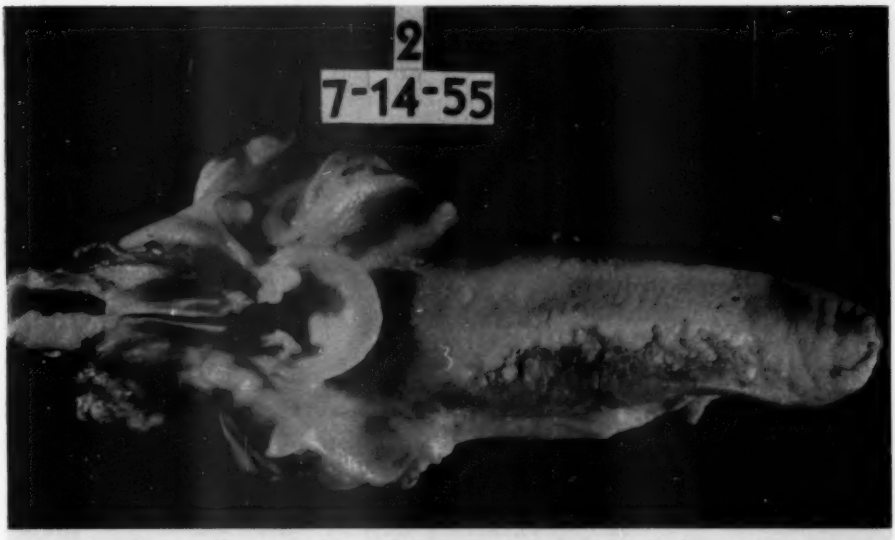


Fig. 9. — Tongue of pig showing oval gross area of ulceration 16 days after exposure to $n+B^{10}$
Neg. No. 7-310-59.

Fig. 10.— Area of margin of ulcer from tongue shown in Figure 9. × 250.
Neg. No. 3-87-58.

cur in the epidermis or whether such alterations are secondary to vascular occlusions in small vessels in the corium, resulting in interference in nutrition. The degenerative and atrophic alterations found in the epidermis of our pigs 25 days after $n+B^{10}$ occurred in focal areas. This was seen in sections from 7 blocks of tissue out of 50 studied in a single irradiated region. The focal character of these epidermal lesions indicates that the tissue response to the irradiation was not uniform. It also suggests that this effect could be a direct one, or at least does not necessarily imply that it occurred secondarily to occlusive vascular lesions of the corium, especially since the latter were not widespread and extensive in these sections.

In man, Linser [3] excised human skin at varying intervals while a patient was receiving x-ray treatment for lupus. He concluded from a serial study of these biopsy specimens that, beginning at 4 days, some vascular channels were occluded by thrombi and that at 30 days many vessels were completely obliterated. Even with severe enough exposure to produce this degree of vascular involvement the epithelium was not secondarily affected. In the skin of the rat, Lushbaugh and associates [4] produced acute dermatitis with 14,000 rep of beta radiation from Sr^{90}. Two days after irradiation the epidermis showed no changes microscopically, but by the fourth day the epidermis was atrophic and an occasional basal cell was swollen. At this time there was slight edema of the dermis, the veins being hyperemic, but there were no degenerative changes in either veins or arteries. By the eighth day small areas of liquefaction necrosis and ulcerations were present in the epidermis. It was not until the 10th day that the walls of the small veins and arteries showed endothelial proliferation, and by the 12th day hyperplastic endothelial and fibrous changes were sufficient almost to obliterate the lumen of some of these channels. Thus, in the experience of these investigators, necrosis, with eschar formation, occurred in the epithelium prior to endothelial proliferation and partial or complete obliteration of vascular channels in the corium. In our pigs epithelial alterations were present at 15 days after irradiation, but how early this actually occurred was not determined.

Another point of interest in the report of Lushbaugh and his colleagues was that by the 14th day after irradiation there were downgrowths from the superficial epithelium, as well as atrophic epithelial remnants in the deep bulbar portion. Mitoses and epithelial "pearls" gave the area an appearance similar to early squamous-cell carcinoma. Hyperplasia of the bulbar-cell epithelium of adjacent relatively undamaged hair follicles seemed to indicate that this appearance was the result of combined upgrowth from the depth of the follicles and the downgrowth of superficial epithelium. In one of our pigs, at 22 days after irradiation, it is felt that the process is a true squamous-cell carcinoma.

Summary

This investigation was undertaken as part of a series on the effects in tissue of uncharged and charged particles of both low and high energy. The pig was selected because its skin, utilized as the tissue of reference, is histologically similar to that of man. The times of killing the animals following irradiation exposure were 15, 24, 25, and 112 days. The B^{10} (n,α) Li^7 reaction was followed by pathologic alterations, characterized by areas of atrophy and of epidermal and dermal necrosis. In one pig, a minute squamous-cell carcinoma was observed in the ear. The animal, killed on the 112th day, had a deep ulcer with a heavy granulation-tissue base, showing vascular changes, such as excessive capillary formation and bizarre endothelial-cell proliferation.

Medical Department, Brookhaven National Laboratory.

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Acute Periarteritis Nodosa with Chronic Glomerulonephritis

Report of a Case

DONALD L. DAWSON, M.D., Denver

The principal renal lesion of periarteritis nodosa is a necrotizing inflammation of small and medium arteries. Ancillary findings include healed arterial lesions, characterized by extensive fibrosis extending from the lumen through all layers of the artery; thrombosis of affected arteries; renal infarction, and extensive fibrosis replacing renal tubules.[1] Arteriosclerosis is regarded as a secondary lesion.[11] An acute proliferative, exudative, or necrotizing glomerulonephritis is commonly associated (Table),

Association of Acute Glomerulonephritis with Periarteritis Nodosa

Author	No. of Cases	No. with Acute Glomerulonephritis
Rose and Spencer[3]	84	33
Spiegel[2]	17	2
Gruber[4]	63	21
Knowles et al.[10]	45	10
Total	209	66

and most authors feel that the association is *causal* rather than *casual*.[1,3,4] Occasionally, subacute glomerulonephritis has been associated with periarteritis nodosa, and in these cases the arterial lesions are both acute and healed.[3,5,6] The association of periarteritis nodosa and chronic glomerulonephritis has been previously reported in but 3 of 84 cases.[2]

The purpose of this report is to record an additional case of chronic glomerulonephritis with superimposed acute periarteritis nodosa.

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Department of Pathology, University of Colorado School of Medicine.

Fellow of the American Cancer Society; partially supported by the Maytag Fund.

Report of Case

A 15-year-old white youth was transferred to Colorado General Hospital on July 28, 1958, because of edema for four months. On March 31, 1958, he had a sore throat and an elevated temperature. Ten days later, he consulted a physician because of red spots on the skin and periorbital and ankle edema. He was treated with prednisone, chlorpheniramine maleate, and ascorbic acid. After a 20 lb. weight gain in one week, he was admitted to another hospital on April 18, where he remained for four weeks, with regression of edema. On admission the urine was dark and contained red cells and albumin. For the next six weeks he was treated at home with a low-salt diet and tetracycline. Because of hypertension, falling hemoglobin, nausea, vomiting, epistaxis, and cardiac failure, he was again hospitalized and treated with chlorpromazine, hydralazine (Apresoline), a digitalis preparation, hexamethonium chloride, thyroid extract, and calcium gluconate. He also received 2,750 cc. of whole blood in 250 cc. units. Oliguria followed two transfusions but responded to administration of hypertonic saline. The last such episode was just prior to his transfer.

Physical examination at the time of transfer showed a blood pressure of 170/120; ascites; severe edema of the extremities, scrotum, and periorbital region; multiple retinal hemorrhages, and slight left-ventricular enlargement. The urine was straw-colored and clear and contained 4+ albumin. A high-power field of the urine sediment contained 50 red blood cells, 40 white blood cells, and an occasional granular cast. The hemoglobin was 9.4 gm. %; the hematocrit, 29%; RBC, 3,700,000; WBC, 9,500, with 89% neutrophils and 10% unsegmented neutrophils. Platelets were normal. Serum sodium measured 128 mEq.; serum potassium, 4.2 mEq., carbon dioxide, 27.3 mEq., chloride, 77.3 mEq., per liter. The blood urea nitrogen was 218 mg. %; creatinine, 14 mg. %. Urine sodium excretion was 7.75 mEq., urine potassium, 36.5 mEq., and urine chloride, 6.5 mEq., in 24 hours.

A left pleural effusion was present. The electrocardiogram showed a rate of 88 per minute; P-R, 0.20; QRS, 0.09; Q-T, 0.36. The ST seg-

ment was depressed, and T-waves were low. T-waves were inverted in Leads V_1 through V_4.

He was initially treated with digitoxin, water restriction, and intravenous hypertonic sodium chloride. On Aug. 4, an antistreptolysin-O titer was greater than 1,000 units. On Aug. 6, hemodialysis was performed uneventfully. On the following day, the temperature rose to 39.2 C; culture of the urine and of the drainage from the femoral cut-down wound showed a hemolytic Staphylococcus, coagulase and mannitol positive; blood cultures were negative; hemoglobin was 2.7 gm. %, hematocrit 8%, and reticulocytes 0.8%. He was treated with penicillin, erythromycin, and packed red blood cell transfusions. Clinical improvement was slow, but definite, until Aug. 15, 1958, when he suddenly developed severe respiratory distress, and diffuse, bubbling rales were heard over the lungs. Despite phlebotomy, theophylline with ethylenediamine, and intravenous lanatoside C, he had a generalized convulsion and died, four and a half months after the onset of disease.

Autopsy, performed 19 hours after death, showed anasarca, bilateral hydrothorax, hydropericardium, ascites, and acute fibrinous pericarditis. The lungs were edematous and showed bronchopneumonia. Other significant pathological findings were confined to the kidneys and blood vessels. The kidneys weighed 460 gm. The renal capsule stripped easily from the smooth, pale-tan surface, marked by dilated, small vessels. The cut surface was convex, and the faintly irregular cortex averaged 4 mm. thick. The brown medullary pyramids were marked by prominent radial blood vessels.

Microscopically, most glomeruli were obliterated, their tubules collapsed, and the adjacent connective tissue relatively increased and infiltrated with lymphocytes. The basement membranes of the remaining glomeruli were thickened, often to the point of occluding capillary loops. The remaining proximal convoluted tubules were lined by low columnar epithelial cells without brush borders. Convoluted tubules were clogged with eosinophilic material, cell debris, and neutrophils. The intima of arterioles was thickened by hyaline material (Fig. 1). Many arcuate arteries were collapsed and the intima destroyed. The internal elastic lamella was irregularly frayed and destroyed. Brilliant eosinophilic material, infiltrated with neutrophils, replaced the media. The adventitia was loosened, contained swollen fibroblasts, and was infiltrated with neutrophils and a few eosinophils and lymphocytes (Figs. 2, 3, 4). Medium and small arteries of the gastrointestinal tract, pancreas, urinary bladder, prostate, and testes were similarly affected. Vessels of the heart and lungs were normal.

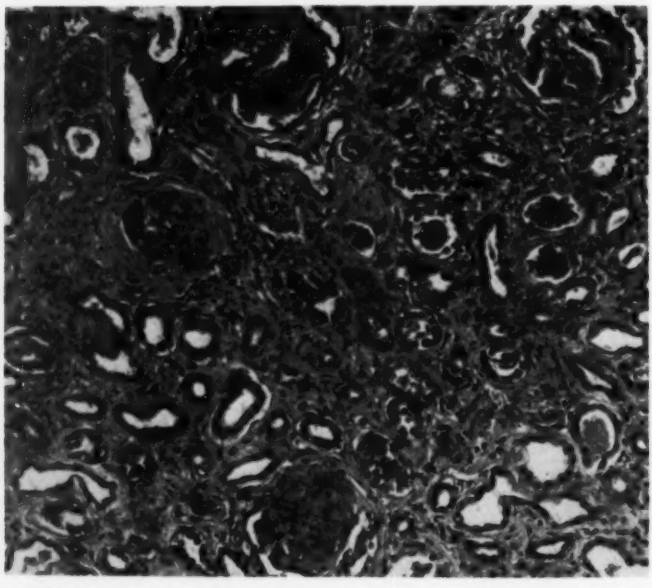

Fig. 1.—Kidney. The glomeruli are obliterated, and interstitial connective tissue is relatively increased. Hematoxylin-eosin stain; mag. × 100.

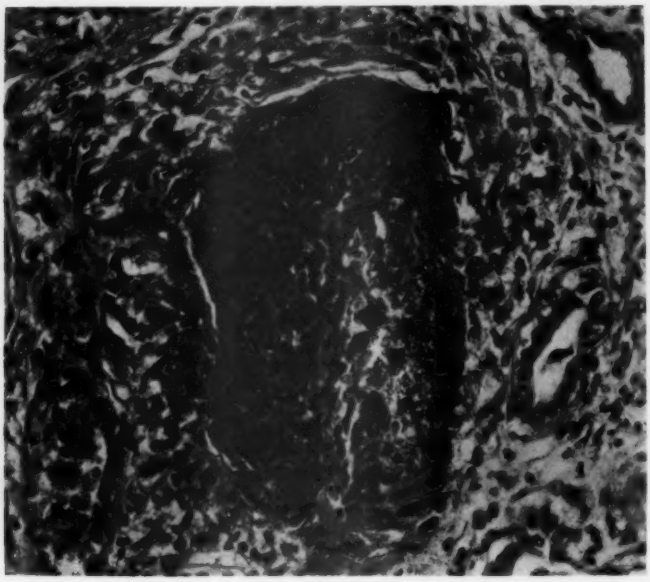

Fig. 2.—Kidney. An arcuate artery with segmental fibrinoid necrosis. Hematoxylin-eosin stain; mag. × 100.

Comment

Since the first descriptions of periarteritis nodosa in 1866, many authors have noted an obscure and inconstant relationship between this disease and serum sickness,[7] renal amyloidosis,[8] rheumatic fever,[9] Loeffler's syndrome (eosinophilic pneumonitis),[9] and glomerulonephritis.[1-6,9,10,16] Where glomerulonephritis occurred, it was usually acute, occasionally subacute, and rarely chronic. Microscopic descriptions of the few chronic cases are not recorded in

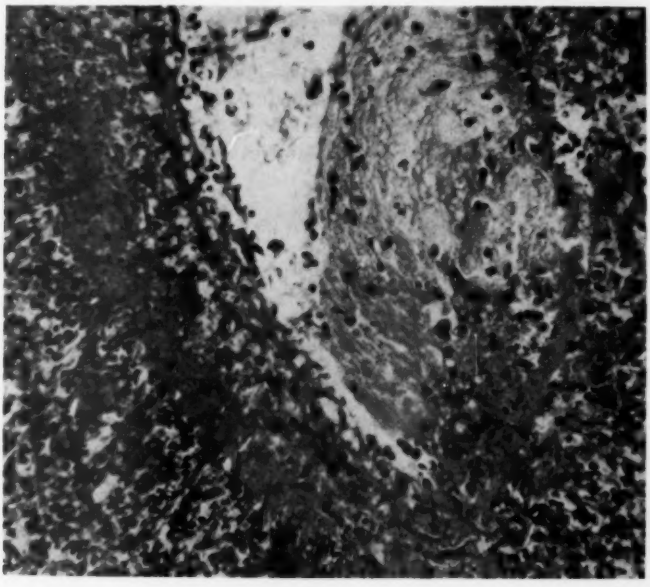

Fig. 3.—Kidney. An arcuate artery, containing a small thrombus, shows necrosis of the arterial wall and marked exudation. Hematoxylin-eosin stain; mag. × 320.

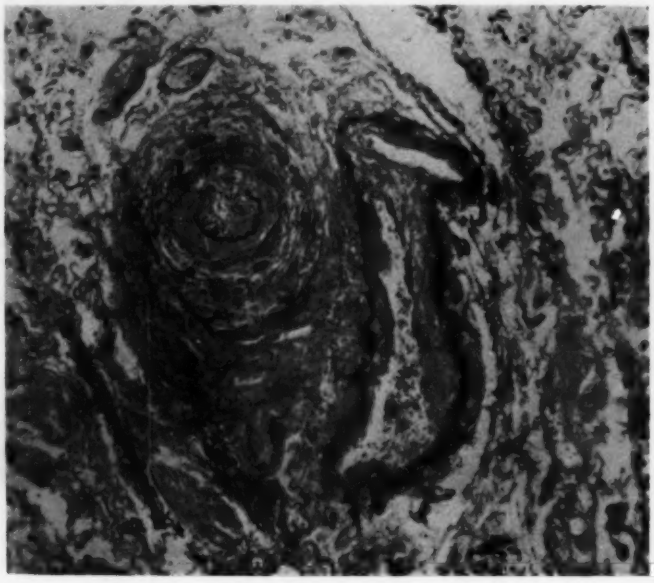

Fig. 4.—Esophagus. The elastica of small arteries is frayed and partly destroyed. Weigert elastic tissue stain; mag. × 10.

detail. Rich [10] was able to produce an acute arterial necrosis in 14 rabbits by a single injection of horse serum, and 9 of these animals also developed an acute diffuse glomerulonephritis. More recently, Mellors [8] demonstrated localization of γ-globulin in the glomerular lesions of human glomerulonephritis and in the arterial lesions of periarteritis nodosa.

In the previously reported associations of periarteritis and glomerulonephritis, the arterial lesions were clearly older than, or of the same age as, the glomerular lesions, and hence a causal relationship was assumed. Acute glomerulonephritis was associated with acute arterial necrosis alone or with the acute lesion and healed arterial lesions. Where subacute or chronic glomerulonephritis was present, many healed arterial lesions were observed, with or without acute arterial necrosis. In the present case, the chronic glomerulonephritis is clearly the older lesion, for only acutely necrotic arteries were observed.

Is there, then, evidence to incriminate glomerulonephritis per se as a lesion necessarily preceding periarteritis nodosa? Certainly, no direct evidence exists. Experiments relating arterial necrosis to hypertension produced by renal manipulation are numerous, and the relationship of these lesions to human disease was reviewed by Zeek.[12] She proposed the generic name "necrotizing angiitis" [12-14] for the group of diseases including periarteritis nodosa and "hypersensitive angiitis." She, and others, have defined clinical [15,16] and pathologic [13,14] criteria for this differentiation. In a statistical review of 175 cases of periarteritis, Nuzum and Nuzum [17] concluded that this differentiation is unwarranted because it appears to divide the acute fulminating cases, which come to autopsy in a few days or weeks, from the more chronic cases, in which the initial response is not as severe and the process is more progressive, with clinical remissions followed by recurrent activity. The time element could explain the more generalized organ involvement in the chronic cases. In any event, although renal damage may be related through hypertension to some cases of experimental necrotizing arteritis, glomerulonephritis per se has not even suggestively been implicated.

Hypersensitivity to a host of therapeutic agents has been frequently postulated as the etiologic factor in periarteritis nodosa. This patient received at least one of these drugs, penicillin. The supposed offenders are sulfonamides,[2,7] diphenylhydantoin (Dilantin),[18] thiourea,[2,19] arsenicals,[2,20] bismuth,[2] iodine,[21] penicillin,[2,22] streptomycin,[2] chloramphenicol,[2] gold,[2] cortisone,[2] and vaccines.[2,7] Rose and Spencer [2] emphasize that to attribute the role of an etiologic agent to a given drug, it must be clearly demonstrated that the disease occurs with greater frequency in a group receiving the drug than may be expected to arise by chance in an untreated population. Such evidence, though admittedly difficult to accumulate, does not exist for most of these agents, the single possible exception being that of the antithyroid drugs. Indeed, from the available evidence, the authors state that the only firm conclusion to be drawn is that "drug hypersensitivity is not a factor in all cases. On the whole [the evidence] favors an abnormal immune response to bacterial infection as the most likely cause in many cases."

Summary

A case of chronic glomerulonephritis showing the acute lesions of periarteritis nodosa at autopsy is reported. In the previously reported association between periarteritis and glomerulonephritis, the arterial lesions were of the same age as or older than the glomerular lesions. In the case reported, the arterial lesions were distinctly acute, as contrasted with the chronicity of the glomerular lesions.

In review of the pertinent clinicopathologic and experimental papers in the literature concerning possible causative factors in the production of the lesions in the kidney of the patient described, the probable sequence of the glomerular lesions was acute, subacute, and chronic in response to an antigen-antibody reaction induced by an initial hypersensitivity to streptococci which caused an upper respiratory infection about 18 weeks before death. As the full-blown chronic glomerulonephritis progressed, acute necrotizing arteritis developed, notably in the kidneys, possibly as a sequal of injury to the renal and other blood vessels by the same antigen-antibody reaction as that related to continued or repeated streptococcal infection and hypersensitivity or to treatment with drugs, such as penicillin, or to both.

Photomicrographs were prepared by Mr. Glenn Mills, of the Department of Visual Education, University of Colorado Medical Center.

University of Colorado Medical Center, 4200 E. Ninth Ave. (20).

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Amyloidosis Induced by Parabiosis in "Genetically Homogeneous" Mice

C. E. HALL, Ph.D.; O. HALL, M.Sc., and E. CROSS, B.S., Galveston, Texas

Subsequent to the report from this laboratory that hypertensive cardiovascular disease can be produced in rats of the Holtzman strain by parabiosis,[1-3] other investigators have found it to be true of Wistar[4] and Slonaker[5] animals. Although attention has been mainly focused on the blood pressure and cardiovascular changes, the basic process is one of extensive collagen disease, which, of over 1,000 pairs studied in this laboratory during the past four years, has affected about 50%. The disorder has always been confined to one of the twins, and the normotensive member has neither contracted the condition through attachment nor been able to prevent the inexorable and fatal progress in its twin. In respect to the hypertensive state, then, the findings are compatible with those of Grollman and Rule,[6] but at variance with those of Braun-Menéndez,[7] with regard to the manner in which renal hypertension is influenced by parabiosis between hypertensive and normotensive animals.

The rat strains prone to develop parabiotic hypertension are also those susceptible to another condition peculiar to the state, that of "parabiosis intoxication," a disorder characterized chiefly by hematologic disturbances.[8-10] Thus it is difficult to decide whether the two conditions reflect a common disturbance, the manifestations depending chiefly on the intensity of the stimulus, or whether they are unrelated, and independently induced by parabiosis. Although hypertensive disease may occur in animals which simultaneously suffer from intoxication, the two conditions as often occur independently.[11] Nevertheless, some investigators have ascribed both the cardiovascular disease and intoxication[5,11] to hypersensitivity phenomena induced by genetic incompatibility.

This laboratory has espoused the view that, although the disturbance fundamental to parabiosis intoxication may well prove to be due to hypersensitivity, its hematologic manifestations are specifically caused by a hemodynamic disequilibrium precipitated by a condition similar in its cardiovascular consequences to traumatic shock.[8,12] However, apparently animals of highly inbred strains do not develop parabiosis intoxication,[13] and it was felt that by using such animals, thus eradicating one of the variables, the two disorders might be separable. This requirement was met by mice of Strain 129 from the Jackson Memorial Laboratory, said by the breeder to be "genetically homogeneous," and these were therefore selected for the study. Since animals of this strain homozygous for the recessive dystrophic gene acquire muscular dystrophy, it was possible simultaneously to learn whether the disorder could be transmitted to a normal co-twin or modified in severity by its presence. These results have been reported elsewhere.[14]

Materials and Methods

Nineteen dystrophic mice were placed in parabiosis, under pentobarbital sodium anesthesia, as soon as the disorder was recognizable, with nondystrophic litter mates of the same sex, using a modified Bunster-Meyer[15] technique. Thirty-one pairs of nondystrophic animals, many of them non-litter-mate pairs, were similarly prepared.

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This study was supported by grants from the National Muscular Dystrophy Research Foundation, Inc., of Liberty, Texas, the National Heart Institute U.S. Public Health Service (H2703), and the American Cancer Society.

Eighteen dystrophic and twenty normal animals served as controls. Pairs and single animals were each individually housed in plastic mouse cages, allowed free access to Purina Laboratory Chow and water, and examined at least weekly thereafter for assessment of general condition. Whenever pairs or dystrophic single animals died or were killed, appropriate controls were killed. The pairs were carefully separated along the anastomosis and the twins weighed individually. Visceral organs were examined for gross lesions, and samples were removed and fixed in Bouin's solution for subsequent histologic examination.

Results

Single dystrophic mice had a life expectancy of 8-10 weeks, and usually no cause for death other than inanition could be found. In parabiosis with normal litter mates, some lived for as long as 37 weeks; but, with very few exceptions, all such pairs died spontaneously. Normal single mice did not die, but were killed to obtain control material. Of the 31 parabiotic pairs of normal mice, 16 died spontaneously; 5 pairs were killed because one or both partners were moribund, and the remaining 10 pairs were killed at the end of one year to complete the data. Mice of Strain 129 are said (by the breeder) to be "genetically homogeneous." This claim was supported by the fact that none of the 50 pairs studied developed parabiosis intoxication, although all of them lived well beyond the period when this syndrome evolves in more heterogeneous susceptible strains.

At autopsy it was evident that of the pairs which had died or had become sick enough to warrant killing, the majority had renal disease. This was apparent in 12 (62%) of the 19 dystrophic-normal pairs and in 19 (91%) of the 21 normal-normal pairs which died spontaneously or were killed because they were moribund. Among the 10 additional pairs of the latter group killed at the end of one year, renal disease was found in only 20%. The over-all incidence in normal pairs was therefore 68%. Mice as young as 2 months of age, in parabiosis for a month, were affected. Cardiac hypertrophy was usually associated with renal disease.

Instances were encountered in which either one or both partners were afflicted, although the extent of the renal involvement was often very dissimilar, either between individuals of affected pairs or as regards individual kidneys of a single affected parabiont. The condition involved both partners in 16 of the 21 affected normal-normal pairs and in 4 of the 12 affected dystrophic-normal twins. Curiously, of the eight pairs of dystrophic-normal parabionts in which only one animal developed lesions, it was the dystrophic partner in seven instances. Incidence in both sexes was the same.

Commonly the damaged kidney was larger than normal, but occasionally it was much contracted and smaller. The contralateral kidney, if not appreciably damaged, was often hypertrophic. In a given kidney the gross lesions were characteristically asymmetrically disposed, and might be few or numerous. Indentations or protuberances of the surface and the acquisition of an irregular contour were typical, and gave to the affected organ a distinctive appearance, which led to the descriptive appellation of "raspberry kidney" (Fig. 1). The heart, though enlarged in such animals, usually bore no grossly visible lesions, nor was involvement of other visceral organs evident. Periarteritis, common in parabiotic rats which develop cardiovascular disease, was not encountered.

Fig. 1.—Hearts and kidneys from a parabiotic pair of mice. Note enlarged heart and distorted kidney contour in those from right partner, affected by amyloidosis.

Histologic Findings

The renal lesions revealed microscopically were variable in both degree of severity and ubiquity, although it was evident that the same basic process was present. In none of the kidneys could lesions properly be termed acute, although clearly they were of more recent onset in some than in others. Cases of apparently short duration usually had enlarged kidneys, partly, as it developed, because of regional hyperplasia of healthy nephrons. In cases of long standing the kidney was usually shrunken, because of extensive atrophy and interstitial fibrosis.

The basic renal lesion appeared to be glomerular. The capillary loops were dilated by an amorphous material, with which, in many cases, they were completely obliterated. In the severest cases this substance infiltrated the interstitial spaces between the tubules of the cortex and invaded the renal papilla. Hyaline material, presumably proteinaceous, often filled Bowman's capsule and the subtending tubule. In some cases virtually all visible glomeruli were affected, whereas in others there were focal accumulations of damaged glomeruli, often in a wedge-shaped pattern, embedded among apparently normal, or even hypertrophic, nephrons. Areas of tubular atrophy and dilation surrounded by hypertrophic elements were common, as was replacement fibrosis. Major blood vessels were not detectably involved. The typical lesions are shown in Figures 2 to 4. In every instance some degree of papillonephritis—or penetration of eosinophilic hyaline material between the collecting ducts, presumed to be antecedent thereto—was observed (Fig. 5). This progressed to actual calcification of the renal papillae in the most chronic cases.

Although undetected by naked-eye inspection at autopsy, microscopic examination revealed the presence of the same amorphous

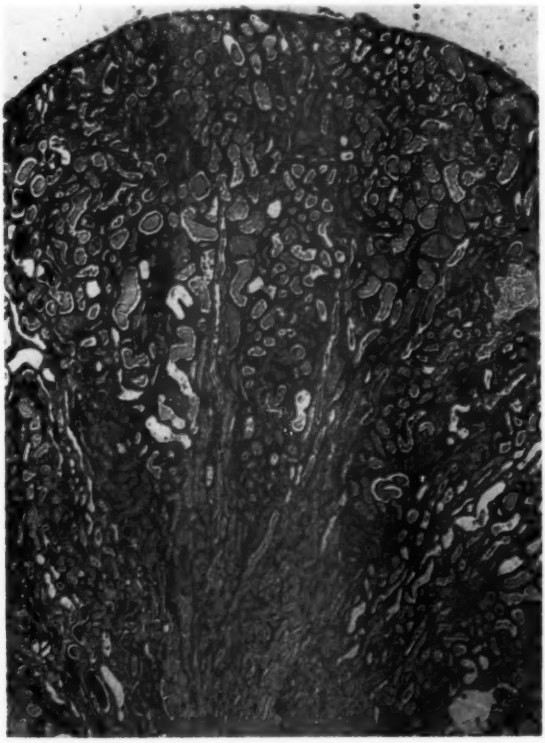

Fig. 2.—Extensive renal amyloid deposition in a 4½-month-old mouse in parabiosis for three months. Hematoxylin-eosin stain; × 40.

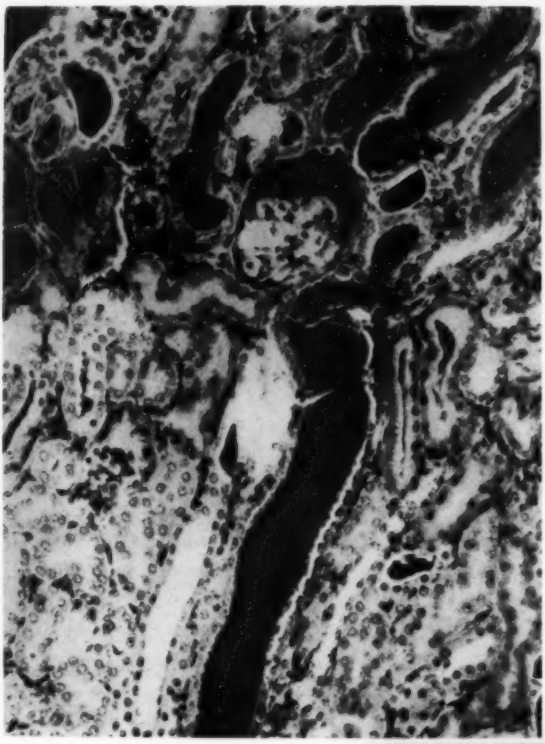

Fig. 3.—Glomerular amyloidosis with proteinaceous material in Bowman's space and tubular lumens from a 3-month-old parabiont, in union seven weeks. Gomori trichrome stain; × 220.

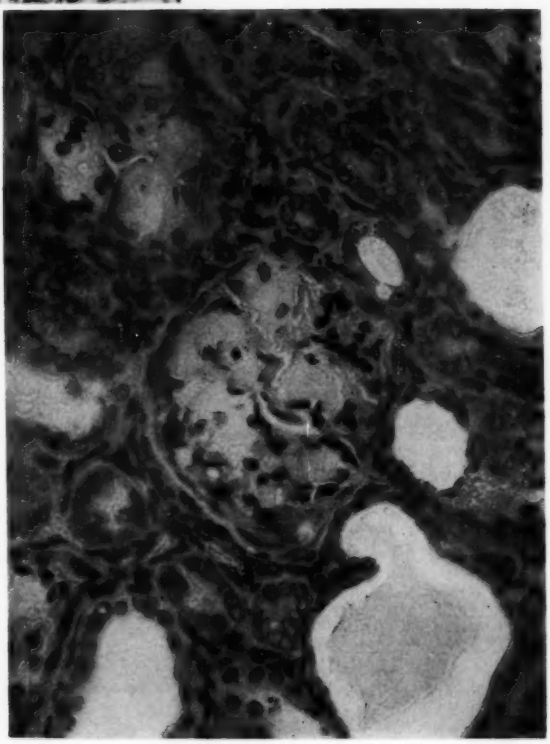

Fig. 4.—Glomerulus completely obliterated by amyloid deposits and cystic dilatation of adjacent renal tubules. Same animal and stain as in Figure 3. × 855.

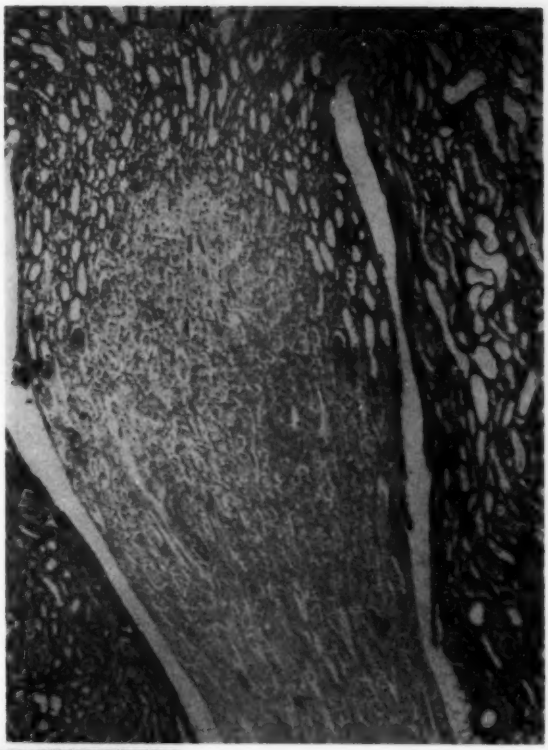

Fig. 5.—Papillonephritis in a 14-month-old parabiont in union for one year. Hematoxylin-eosin stain; × 51.

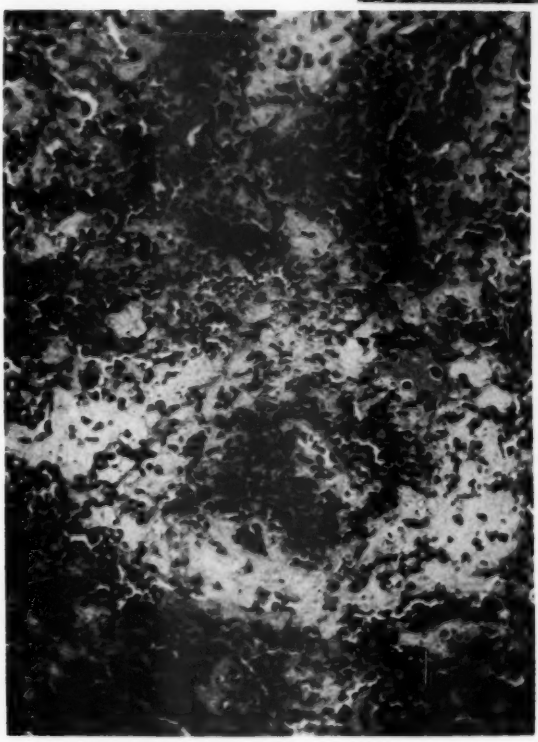

Fig. 6.—Amyloid infiltration of spleen. Same animal as in Figure 4. Gomori trichrome stain; × 460.

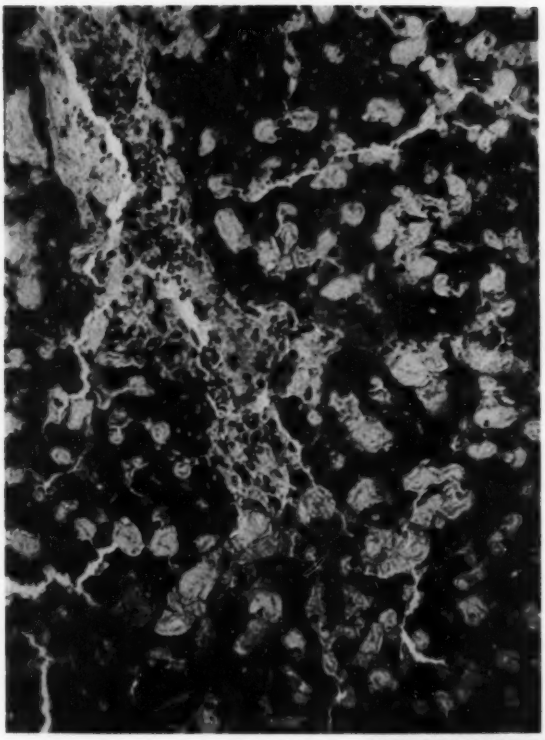

Fig. 7.—Extensive hepatic amyloid infiltration in a 4-month-old parabiont, in union for 10 weeks. Gomori trichrome stain; × 440.

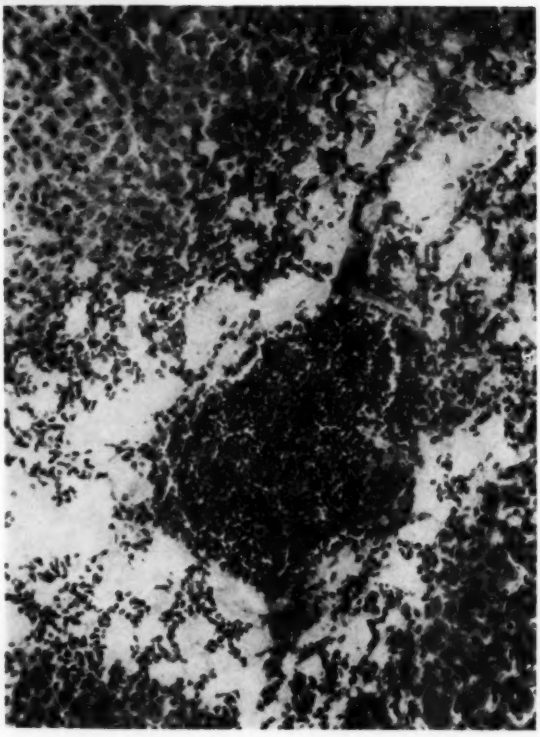

Fig. 8.—Replacement of adrenal medulla by amyloid deposits and beginning invasion of cortical reticularis in a 4-month-old mouse in parabiosis for 12 weeks. Gomori trichrome stain; × 200.

substance in other organs. In decreasing order of frequency, although in some cases all were involved, lesions were seen in the spleen and kidney, liver, adrenals, pancreas, and heart. The spleen and kidney were invariably affected; the liver, in about 90% of the cases, and the adrenals, in approximately 75%. The incidence of pancreatic involvement was about 60% in those samples available for examination. Other viscera, except for the heart (to be described later), were not examined.

In the spleen the material tended to invade in sheet-like masses, displacing the lymphoid elements, beginning at the periphery of the Malpighian bodies but never confined to them (Fig. 6). In the liver the material was detected in the media of veins and in the walls of peripheral sinusoids, and was insinuated between hepatic cells, which it tended to crowd out, forming confluent and contiguous masses of ever-increasing size (Fig. 7). Adrenal involvement, when present, was almost always evident in the medulla and in the cortical reticularis, frequently in the fasciculata, and very rarely in the glomerulosa. The material invaded intracellularly, tending to replace the medulla and displace the rows of cortical cells (Fig. 8). In the pancreas, both acini and islets were pervaded, and often almost completely replaced, by hyaline material (Fig. 9). The capillaries and smaller veins were particularly affected.

The hearts of animals with renal lesions were often only hypertrophied, although not uncommonly extensive myocarditis or fibrosis was present (Fig. 10). A curious finding was that with the Gomori trichrome stain, in many cases some fibers stained brick-red and others with the contrasting stains. The basis for this curious tinctorial response is unknown, but is has been reported to occur in human amyloidosis.[16] The red-staining myocardial fibers had an amorphous hyaline appearance; the nucleus was small, compact, and densely black, rather than vesicular, as in the normal fibers. Here, and occasionally in the other involved tissues, cushion-like swellings of venous walls, presumably due to amyloid infiltration, were sometimes observed (Fig. 11).

The amorphous material was found to stain pink with eosin, red with the periodic acid-Schiff stain, lightly pink with crystal violet, and green with light green. Attempts, in a few cases, to stain with Congo red were unsuccessful. No such lesions were

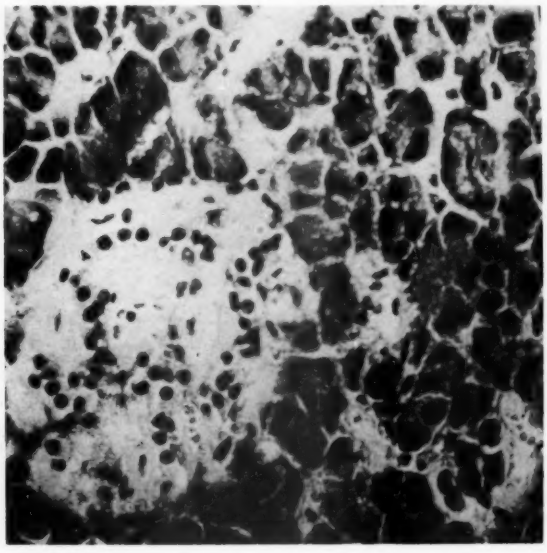

Fig. 9.—Amyloid infiltration of acini and islet of Langerhans in a 14-month-old parabiont, in union for one year. Gomori trichrome stain; × 807.

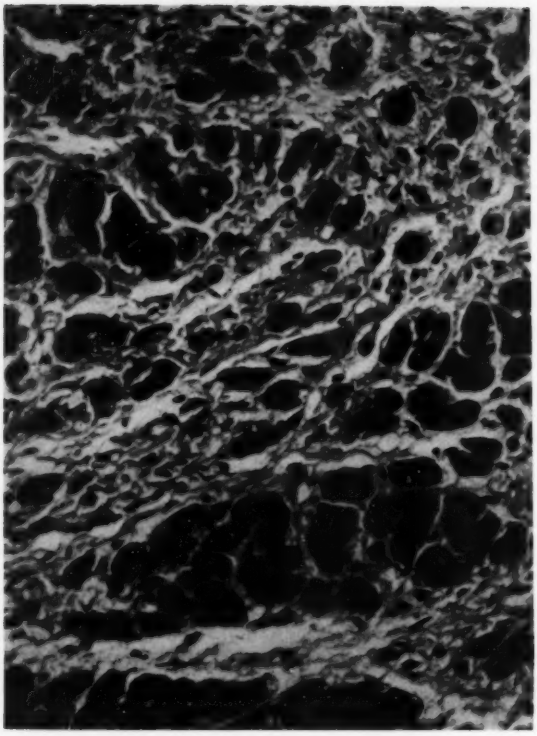

Fig. 10.—Extensive fibrosis and amyloidosis in the heart in an 11-month-old parabiont, in union for eight months. Gomori trichrome stain; × 380.

encountered among single control animals, even those with extreme cachexia, which succumbed to muscular dystrophy.

Comment

In view of the staining properties, some remarks on the diagnosis of amyloid seems

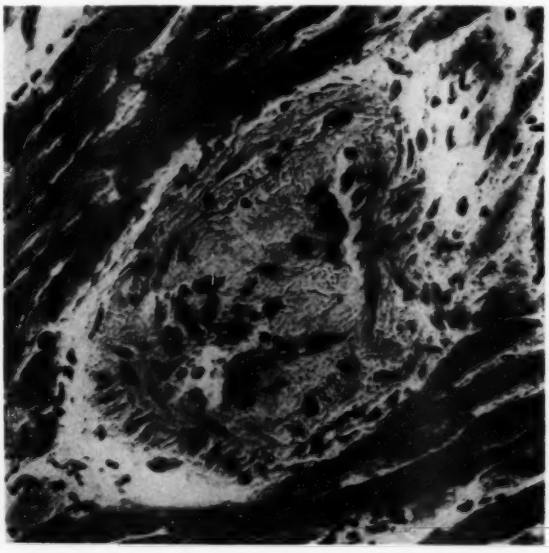

Fig. 11.—Intramural amyloid in a cardiac vein, from a mouse 8½ months old, in parabiosis seven months. Gomori trichrome stain; × 300.

warranted. Many different classifications of amyloidosis have been proposed, none being entirely satisfactory because of transitions between the categories established. The staining characteristics are notoriously fickle, presumably because amyloid is not a specific chemical entity but is composed of a number of closely related proteins (paraproteins), tinctorially related to Bence Jones protein.[17] So-called "primary" amyloidosis frequently has the same organ distribution as "secondary" amyloidosis,[18] thus raising the question as to the justification for such arbitrary separation,[19] particularly when the cause of the amyloid deposition is unknown in either instance.[20]

The tinctorial identification of amyloid in the mouse has in general been even more difficult than in the human. Their failure to acquire the expected stains led Gorer and associates [21] to describe as "hyaline disease," renal lesions in the mouse which four years later were diagnosed as amyloid.[22] The problem of arriving at a certain diagnosis has been commented upon by Dunn,[22] Turnbull,[23] and Thung.[24] The consensus seems to justify the term "mouse amyloid," [23] and it has been pointed out not only that histologic criteria are superior to tinctorial niceties in establishing the diagnosis in an area where a discriminating nomenclature has not been established, but that, if the histologic criteria, despite obvious similarity to those of human amyloidosis, are rejected as inadequate, the process in the mouse must then be regarded as one having no known analogue in human pathology.[24] In the present experiment the histologic appearance of the infiltrating material was identical with, and the distribution compatible with, that reported as mouse amyloid by others,[22,24,25] even producing the characteristic papillonephritis.[22] Furthermore, it did, in some instances, show metachromasia with crystal violet.

Inasmuch as amyloidosis may be induced in mice by a variety of apparently unrelated procedures,[26-29] and even occurs spontaneously in the older mice of some strains,[5,25] the reasons for concluding that parabiotic amyloidosis is an experimental form require elaboration.

The failure to encounter the disease in such a large number of single animals of up to 13 months of age, even in those dying with the severe cachexia of dystrophia muscularis, suggests that Strain 129 is not particularly susceptible to spontaneous amyloidosis. There are no infallible criteria by means of which spontaneous, or "primary," can be distinguished from experimental, or "secondary," amyloidosis in the mouse. However, Dunn [30] has mentioned that renal amyloidosis, which simultaneously affects the glomeruli, the papillae, and the intertubular areas of the cortex, is especially prominent, as in the present experiment, in the secondary form of the disease. Then, too, in his discussion of the characteristics of senile amyloidosis in several strains of mice, Thung [25] mentions that whereas the acini in the pancreas were commonly involved, amyloid was invariably absent from the islets of Langerhans. Islet amyloid was present in the parabiotic disease. The available evidence thus favors experimental amyloidosis. Since the pattern of distribution which initially tends to differentiate primary from secondary amyloidosis in the mouse tends to become uniform in the chronic form of either,[24] even though the single Strain 129 mice examined have not shown evidence of spontaneous amyloidosis, it is perhaps wiser to regard the experimental disease as resulting from the acceleration of a process which slowly produces senile amyloidosis in some mice. It seems also reasonable to suppose that widespread amyloidosis might indicate not only a long-continued process but also an intensified reaction. Parabiosis might thus lead to the rapid extension of amyloid from one organ to another, whereas other experimental means of causing amyloid formation might require a longer period to obtain the same extent of involvement.

Why does parabiosis cause amyloid disease? Bacterial cultures,[26] casein ad-

ministration,[27] gelatin, egg protein, and colloidal sulfur[28] have each been found capable of causing amyloidosis in mice. Letterer[31] has implicated disturbed antibody-antigen relationships, and others have suggested as causative either the exhaustion of antibody-forming capacity of the reticuloendothelial cells[32] or an alteration in the normal metabolism of plasma cells.[33] The possibility that amyloidosis may be the end-result of a slowly developing hypersensitivity reaction has been entertained,[34] and it has been suggested that both collagen disease and amyloidosis are related allergic hyaline diseases, distinguished chiefly by the rapid evolution and necrosis found in the former and the slow evolution and lack of necrosis in the latter.[34] This possibility is attractive if for no other reason than that parabiosis produces amyloidosis in the mouse and collagen disease in the rat.[1,5,35] In man, also, hypersensitivity to sulfonamide may induce either collagen disease[36] or paramyloidosis,[37] and it is interesting that the same agents which provoke amyloidosis in susceptible species (foreign sera, microorganisms, sulfonamide drugs, etc.) have also been found to cause periarteritis nodosa.

Preliminary experiments with Swiss Albino mice indicate that these also develop amyloidosis when placed in parabiosis. However, their genetic heterogeneity produces a high mortality from parabiosis intoxication. This contraindicates their use in such studies but does demonstrate that parabiotic amyloidosis is not unique to Strain 129.

Parabiosis, by its very nature, creates circumstances favorable to the development of hypersensitivity reactions should either partner have circulating antibodies, either naturally occurring or induced, against blood-borne antigens of the twin. Because of this, and the experimental evidence implicating the antibody-antigen response in amyloid formation, it is tempting to think of parabiotic amyloidosis as being due to a hypersensitivity reaction, perhaps attributable to plasma proteins, a view which harmonizes with the finding that the injection of homologous serum globulin produces amyloidosis in the rabbit.[38] It has even been suggested that mouse amyloid results from a functional disturbance of the mesenchyme as a part of the stress syndrome,[39] a view which could be easily reconciled with the stress of parabiosis, as has already been pointed out in respect to parabiotic collagen disease in the rat.[1] The unusual sensitivity of mice to amyloidosis, however, dictates caution in the formulation of hypotheses as to causation and indicates the need for further investigation before definite conclusions are reached. It is quite evident, however, that mere "genetic homogeneity," which effectively prevents the occurrence of parabiosis intoxication, offers no immunity to development of amyloidosis.

Although the percentage of pairs affected by amyloid disease was 68% in the case of parabiosis between normal animals and 62% in parabiosis between dystrophic-normal pairs, it tended, when present, to affect both partners in the former group and more commonly only one of them in the latter. Hence, if the numbers of affected mice, rather than pairs, are compared, the incidences were, respectively, 63% and 42%. The extent to which longer average survival of the pairs or the circumstance of non-litter-mate pairing may have contributed to the greater incidence among normal parabionts is uncertain.

Summary

When mice of Strain 129 are placed in parabiosis, amyloidosis develops in 60%-70% of the pairs, in some instances within a month of union. The disease may affect both members of the pair or be restricted to one of them. Those with the most widespread involvement usually die spontaneously, although the disorder was discovered in some apparently healthy animals killed after a year in union. Not all tissues have been examined; but when those which have are listed in decreasing order of frequency and severity, amyloidosis is

found to have affected kidneys and spleen, liver, adrenals, pancreas, and heart. Tinctorial characteristics of mouse amyloid are described, and circumstances which may be involved in its formation are discussed. Instances of "parabiosis intoxication" have not been observed among pairs of this strain, supporting the purported "genetic homogeneity."

Department of Physiology, University of Texas Medical Branch.

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Lupoid Hepatitis with Advanced Atrophic Cirrhosis

Review of the Literature and Report of a Case

JOHN D. KING, M.D., Kansas City, Mo.

Hepatic lesions are quite common in systemic lupus erythematosus but are usually so mild as to attract little attention. As high as a 52% incidence of hepatomegaly has been recorded in one series of cases.[21] Of the large series (138) cases) of Harvey and co-workers,[15] hepatomegaly was discovered during life in 35%, and was described as a "common" finding at autopsy. Other series of directly observed cases[7,13,18,20,24] show a 25% to 39% incidence of liver enlargement in systemic lupus erythematosus. Histologically, fatty change is common,[13,15,20] and centrolobular atrophy, and even mild focal scattered necroses, are seen not infrequently, without significant clinical or laboratory signs of liver disease.[13,15,18,20,21] Other findings have included small focal granulomas,[21] thrombi,[7,20,27] arteritis,[7,13] inflammatory infiltrate,[13] submucosal edema of the biliary ducts, the gallbladder, and the ampulla of Vater,[15] and marked proliferation of Kupffer cells.[20]

It is these cases of systemic lupus erythematosis with minor liver damage that Mackay and co-workers [23] have referred to as "hepatic lupus." The instances of severer liver damage accompanied by positive L.E. cell tests they have designated "lupoid hepatitis."

A total of 25 cases qualifying as lupoid hepatitis have been reported in some detail. Eighteen [1,2,12,19,23,24] have had histologically confirmed hepatitis and/or cirrhosis, in addition to a clinical and laboratory picture suggesting systemic lupus erythematosus, including an elevated serum globulin, especially γ-globulin, abnormal urinary sediment, leukopenia, and in all cases at least one positive L.E. cell test. Of these 18 cases, only 6 had any histologic confirmation of systemic lupus erythematosus whatever, and much of this was nonspecific and scanty. The histologic findings in the liver consisted in the main of focal or diffuse hepatic necrosis, regenerating hepatic lobules separated by fibrous tissue and parenchymal atrophy, and round-cell infiltration. In all cases there was jaundice or chemical derangements compatible with liver damage, e.g., elevated serum alkaline phosphatase, increased sulfobromophthalein (Bromsulphalein) retention, and elevated serum bilirubin. Increased serum flocculation tests were common but in themselves not at all diagnostic of liver damage, since the globulin changes of systemic lupus erythematosus so frequently produce the same results without significant liver damage.

Seven cases [19,23,25,32] have been reported in which L.E. cells were found in patients with histologically proved active chronic hepatitis and/or cirrhosis, but in the absence of a clinical picture or other evidence of systemic lupus erythematosus.

In addition, one case [21] is reported of a patient, also without signs of systemic lupus erythematosus other than a positive L.E. cell test, but with marked hyperbilirubinemia and hepatomegaly. At autopsy focal granulomas and severe bile stasis in the liver were seen without either hepatitis or portal cirrhosis.

There are several other reports [1,17,21] of patients with positive L.E. cell tests accompanied by putative hepatitis and cirrhosis;

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Department of Pathology, St. Luke's Hospital.

however, liver disease in these cases was not histologically confirmed.

Bearn et al.[2], have reported 26 cases of cirrhosis of the liver in women. Eleven, or 42%, had joint pains, and three had chronic joint changes similar to those of rheumatoid arthritis. All these patients had extraordinarily high serum γ-globulin findings. However, only one had L.E. cells. Sherlock [31] alludes to atypical cirrhosis in young women, accompanied by hypergammaglobulinemia, hyperadrenocorticism, and occasionally positive L.E. cell tests. She observed three such cases, but failed to state whether these three produced L.E. cells. Cirrhosis of the liver was reported in three of Harvey and co-workers' cases of systemic lupus erythematosus, but the details of these cases were not specified.[15]

The consideration of the L.E. cell phenomenon immediately raises the question of its specificity. Although L.E. cells appear in the great majority of cases of systemic lupus erythematosus, they have been reported in pernicious anemia,[3,4] dermatitis herpetiformis,[3] chronic discoid lupus erythematosus,[3] leukemia,[34] multiple myeloma,[14,22] hypersensitivity to hydralazine [8,26,28,30] and penicillin,[35] and chronic systemic moniliasis,[11] and even in apparently healthy persons.[5]

It should be pointed out that the cells designated "L.E." by various authors may not in fact be the same cell, inasmuch as its definite identification is sometimes difficult. Reputed L.E. cells in a case of primary amyloidosis were later shown probably to be cells with ingested amyloid.[22] Even contamination of equipment could conceivably account for false-positive readings. Haserick,[16] for example, reported formation of cells "closely resembling" L.E. cells in the presence of a certain strain of Aspergillus niger. Furthermore, systemic lupus erythematosus often pursues a long, intermittent course, and some of the so-called false-positive L.E. tests cited above may eventually prove to have been latent or otherwise unrecognized cases of true systemic lupus erythematosus.

With these reservations in mind, it appears probable that L.E. cells are produced in conditions other than systemic lupus erythematosus, but such occurrences probably are rare.[15,19,22]

Severe liver disease with concomitant signs and symptoms of systemic lupus erythematosus, or simply a positive L.E. cell test, suggests the following possible relationships:

1. Hepatitis and cirrhosis may constitute infrequent visceral lesions of systemic lupus erythematosus. The frequency of vascular lesions in systemic lupus erythematosus, together with the great vascularity of the liver, makes such a relationship feasible. Vascular lesions in the liver suggestive of systemic lupus erythematosus have been described.[7,15] However, hepatic vascular lesions are not in the least apparent in most cases of either lupoid cirrhosis or hepatic lupus.

2. Systemic lupus erythematosus may occur purely fortuitously with cirrhosis or hepatitis. Such an explanation cannot be disputed in any single case or small group, but does seem unlikely for the number of cases reported. Acidophilic bodies similar to those reported in viral hepatitis were seen in only one case.[1]

3. Virus or other hepatotoxic agents may attack not only the liver but other organs as well, producing hepatitis with the additional features usually seen in systemic lupus erythematosus. The infrequency of any manifestations of systemic lupus erythematosus with hepatitis or cirrhosis, and the present knowledge of known hepatotoxins make direct extrahepatic actions of these agents seem highly improbable.

4. Poor nutrition and other secondary constitutional aspects of a wasting disease, such as systemic lupus erythematosus, may cause liver damage of a nutritional nature. Such a mechanism seems a reasonable explanation, at least in part, for the common minor liver damage observed in systemic lupus erythematosus (hepatic lupus), and undeniably could play an accessory role in true lupoid hepatitis. However, there is no

apparent evidence that the occurrence of full-blown hepatitis or cirrhosis corresponds to the degree of debilitation or malnutrition.

5. Both chronic liver disease and systemic lupus erythematosus may result from autoimmune antibodies. This explanation at present seems the most adequate.

Joske and King [19] suggest that the L.E. cell phenomenon is indicative of the presence of autoantibodies to leukocytes and might occur in any condition in which abnormal antibodies are produced. The autoantibodies could result from transformation of a body component into an antigen or by a disorder in the antibody-producing mechanism. They suggest that alteration of liver proteins by virus or other injurious agents could thus stimulate the reticuloendothelial system to produce antibodies to the altered hepatic proteins and, on occasion, to leukocytes as well. Identical or similar antibodies might, in addition, attack other tissues, e.g., vessels of skin and joints, to produce features of systemic lupus erythematosus.[6] The reported occurrence of L.E. cells and syndromes simulating systemic lupus erythematosis in hypersensitivity reactions lends support to such a view, as does occurrence of various hematologic abnormalities in systemic lupus erythematosus.[6,17] Eaton, Murphy, and Hanford [9] noted complement-fixing antibodies in acute hepatitis and suggested, as did others subsequently,[22,23,27,36] that prolonged hepatitis had features of a hypersensitivity reaction. Spellberg [32] suggests that the L.E. cell occurring in liver disease with marked hypergammaglobulinemia represents an unusual antigen-antibody reaction.

Gajdusek [10] recently demonstrated autoimmune complement-fixing reactions in some cases of acute and chronic viral hepatitis and systemic lupus erythematosus. The reactions became negative in the hepatitis patients who completely recovered, but remained positive in the patients with chronic hepatitis and systemic lupus erythematosus. Using the serum of one of their patients with lupoid hepatitis (Case 2),[23] Taft et al.[33] obtained serum complement-fixing reactions against a variety of human tissues. Most significantly, the titers were substantially higher against the patient's own tissues than against those of other persons.

Report of Case

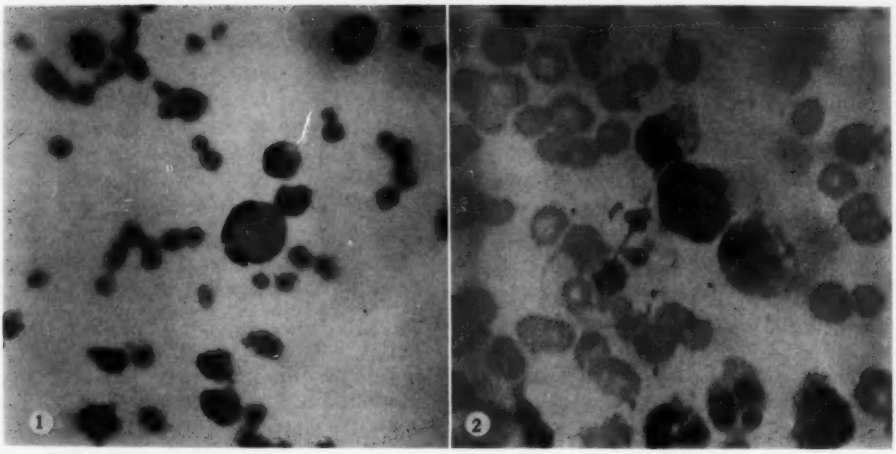
The patient, a 65-year-old white woman, was admitted to St. Luke's Hospital on April 10, 1954, because of fatigue, vomiting, and afternoon fever of about one month's duration, and dyspnea and pedal edema for about one week.

The past history included persistent conjunctivitis in 1939; tonsillectomy for chronic tonsillitis in 1940; a patchy, dry, scaling erythematous rash of the palms, neck, and trunk of several months' duration in 1946, and discovery of a "slightly enlarged," nontender liver the following year. For many years she had complained of "indigestion," i.e., flatulence and cramping abdominal pain, and mild arthritic pain in the fingers.

In December, 1953, the patient underwent cholecystectomy because of gallstones. The liver at that time appeared grossly cirrhotic and the spleen moderately enlarged; liver biopsy was not done. There was no history of alcohol intake.

On her final admission, the patient was noted to be an elderly white woman appearing chronically ill. The venous plexus of Sappey was markedly engorged. The abdomen was rather protuberant, and a fluid wave was questionably elicited. Neither the liver nor the spleen was palpable. No joint deformities were observed. There was mild pitting edema of the lower extremities.

Laboratory Data.—Hemoglobin 8.5 to 11.5 gm. per 100 ml.; erythrocytes 2,800,000 to 3,800,000 per cubic millimeter; leukocytes 5,150 to 23,700 per cubic millimeter, all differential counts showing a marked shift to the left. Numerous urinalyses showed a trace to a 1+ reaction for albumin, many pus cells, and usually positive benzidine tests, although erythrocytes were never observed microscopically. During the fourth week, nonprotein nitrogen was 43.5 mg. per 100 ml., and creatinine, 2.2 mg. per 100 ml.; prior values had been within normal limits. Total serum protein was 6.95 to 9.25 gm. per 100 ml.; albumin, 2.06 to 3.30 gm. per 100 ml., and globulin 4.36 to 7.05 gm. per 100 ml. The A/G ratio was 0.31 to 0.51. Electrophoresis was not done. The icterus index was 18.1 to 57.3. Serum bilirubin values were not obtained. Serum cholesterol was 80 to 100 mg. per 100 ml., with 65% esters. Erythrocyte sedimentation rate (Wintrobe) was 18 to 26 mm. in one hour. One cephalin flocculation test was 4+ in 24 hours. One prothrombin-time determination was 79%.



Figs. 1 and 2.—L.E. cells prepared from clotted peripheral blood. Wright's stain; reduced to 57% of mag. × 970.

Two L.E. clot tests on venous blood both produced large numbers of L.E. cells (Figs. 1 and 2).

Through most of her hospital stay, the patient had nausea and vomiting, bright and dark blood being observed in the vomitus on occasion. Beginning about the second month, the patient had episodes of mental confusion, progressing to stupor and intermittent coma within three weeks. She complained of severe pain in the arms and legs, but no localization to or swelling of the joints was observed. There was progressive increase in the pitting edema of the lower extremities. Jaundice appeared soon after admission and progressively deepened. She had intermittent fever, with a temperature up to 102.2 F (oral). She gradually became weaker and died May 29, 1954, on the 59th hospital day. Therapy included sedatives, codeine, mercurial diuretics, penicillin, almost daily I.V. fluids, and 3 pt. (1,500 cc.) of whole blood.

Autopsy was performed 5 hours and 50 minutes after death. The body was not embalmed. There was moderate jaundice. Pitting edema of the lower extremities was present. The liver weighed 800 gm. Its external surface was found to be nodular, the nodules not exceeding 4 mm. in diameter. The cut surface showed the same nodular pattern, and was firm and grayish-brown. The gallbladder was absent, and the greater omentum was adherent to its bed. The extrahepatic bile passages were patent and unscarred. The spleen weighed 350 gm. and was of normal consistency and color. The lower part of the esophagus contained several varices; the gastrointestinal tract was otherwise not remarkable. The peritoneal, pericardial, and pleural cavities contained a normal amount of clear, yellow fluid. No joint deformity was noted. The cardiovascular system was devoid of gross lesions except for mild aortic atherosclerosis. The kidneys were not grossly noteworthy.

Microscopically, the liver showed marked atrophic cirrhosis, with perilobular fibrosis, lymphocytic infiltration, some bile-duct proliferation, and both degeneration and regeneration of hepatic cord cells (Fig. 3). In the glomerular tufts of the kidneys were focal areas of basement-membrane thickening, somewhat suggesting a "wire loop" (Fig. 4). No definite splenic periarterial lesions were seen. The lungs showed areas of atelectasis, early bronchopneumonia, and some intra-alveolar edema fluid. Esophageal varices were microscopically confirmed.

Comment.—This case lacks the clinical history, laboratory data, and clear-cut histologic findings necessary for an unequivocal diagnosis of systemic lupus erythematosus. We believe that it definitely does represent a case of lupoid hepatitis, an entity whose only two essential features are chronic hepatitis and/or cirrhosis and the L.E. factor. Whether the history of joint pains and the skin rash, and the kidney lesions discovered at autopsy, are indicative of true systemic lupus erythematosus is a matter for speculation.

Summary

Relatively minor degrees of liver damage occur frequently in systemic lupus erythematosus. Twenty-five cases of histologically confirmed hepatitis and/or cirrhosis with associated positive L.E. cell tests have been reported, the disease in these cases being

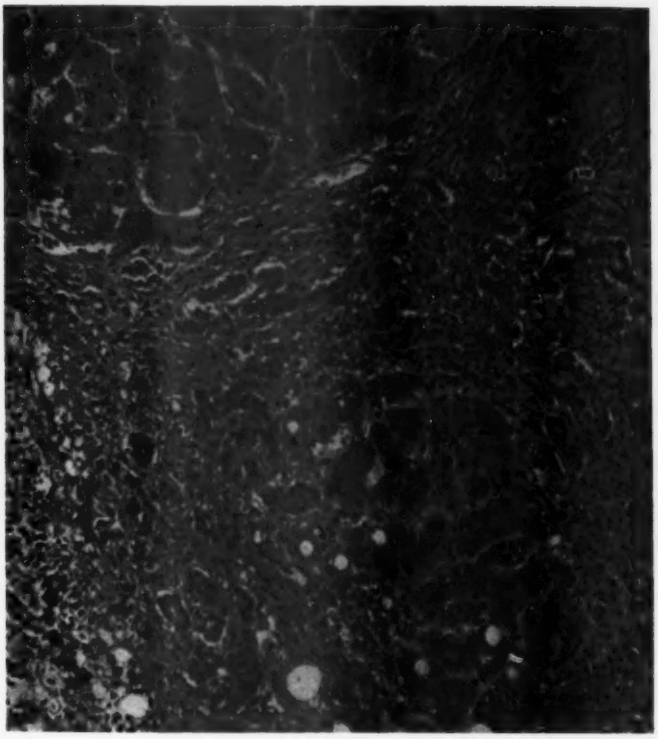


Fig. 3.—Liver showing distorted lobules with perilobular fibrosis, lymphocytic infiltration, and bile-duct proliferation. Hematoxylin and eosin stain; reduced to 88% of mag. × 430.

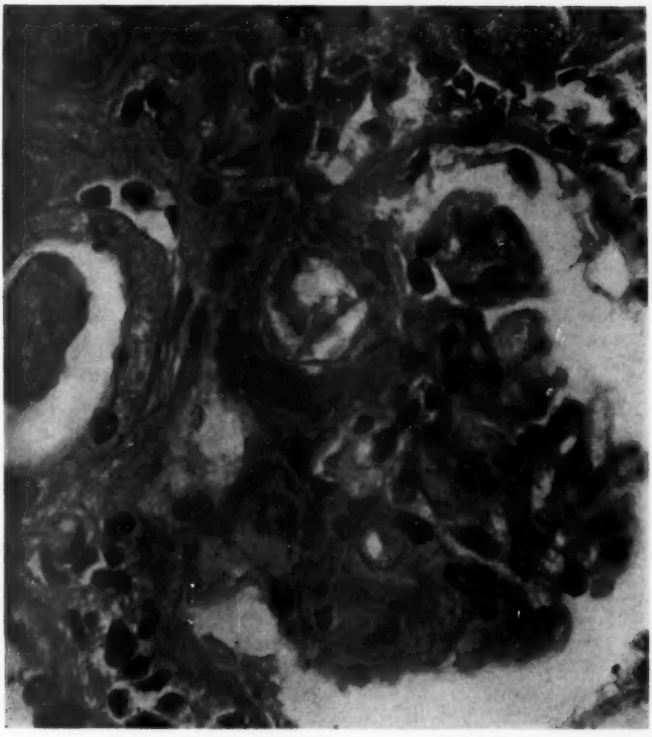


Fig. 4.—Kidney glomerulus with hyaline band resembling a "wire loop." Hematoxylin and eosin stain; reduced to 88% of mag. × 430.

designated as lupoid hepatitis. Of the 25, 18 cases had a clinical course suggestive of systemic lupus erythematosus, although pathologic diagnosis of the full-blown disease was lacking in most of the cases.

Several explanations of the pathogenesis of lupoid hepatitis and cirrhosis are considered. The most likely in the light of present knowledge is that L.E.-cell formation, systemic lupus erythematosus, and chronic hepatitis and its resulting cirrhosis are all expressions of autoimmune reactions. The antibodies involved may sometimes act in a multiple fashion to produce lupoid hepatitis.

The case of a woman with histologically proved chronic hepatitis and portal cirrhosis with renal glomerular lesions and an associated L.E.-cell phenomenon is reported.

I wish to thank Dr. Maxwell G. Berry for permission to publish this case.

Department of Pathology & Research, St. Luke's Hospital, 4400 J. C. Nichols Pkwy. (11).

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Fibroadenoma of Gallbladder

Report of Case with Cholecystographic Visualization

SEYMOUR FISKE OCHSNER, M.D., and G. M. CARRERA, M.D., New Orleans

The following case is reported because it is believed to represent a type of neoplasm of the gallbladder hitherto unreported. The tumor, a fibroadenoma, was visualized as a fixed filling defect in cholecystograms, and in the resected gallbladder it appeared as a pedunculated lesion in the fundus.

Report of Case

A lawyer, 47 years old, was seen at the Ochsner Clinic on July 17, 1957, complaining of recurrent attacks of abdominal cramps and pain in the right upper abdominal quadrant for seven years. These attacks occurred chiefly after meals and were associated with indigestion, belching, and gaseous distention. At times the pain extended to the right subscapular area. The attacks were not associated with fever, chills, or jaundice.

The gallbladder had been examined roentgenographically on five occasions between 1950 and 1957.* The earliest one, on Jan. 10, 1950, showed a small radiolucent defect, measuring 3×5 mm., near the tip of the fundus (Fig. 1). In the cholecystogram of May 11, 1955, the radiolucent shadow had enlarged slightly, measuring 6 mm. (Fig. 2). In the roentgenogram of June 18, 1957, the shadow was unchanged (Fig. 3). The radiolucency was fixed in relation to the wall of the gallbladder and was considered preoperatively to be a polypoid mucosal tumor, or possibly an embedded gallstone.

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From the Departments of Radiology (Dr. Ochsner) and Pathology (Dr. Carrera), The Ochsner Clinic.

* These films were made available for study by Dr. Gayden Ward, of Jackson, Miss.

Fig. 1.—Cholecystogram made Jan. 10, 1950, shows 5×3 mm. radiolucent shadow in the fundus of the gallbladder.

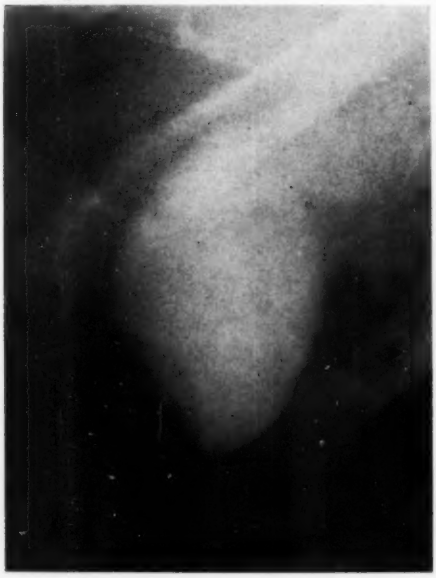
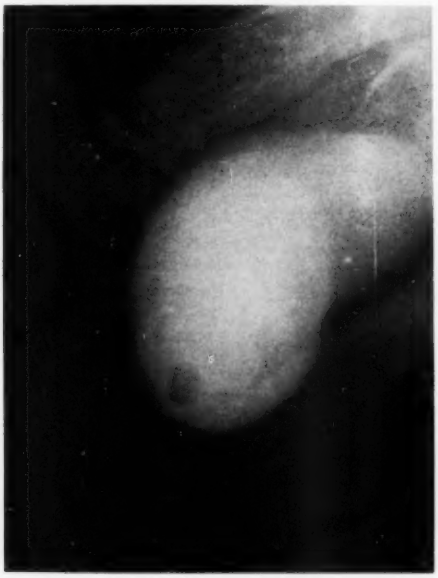


Fig. 2.—Cholecystogram made May 10, 1955, again shows the fixed radiolucent shadow, which has increased to a diameter of 6 mm.



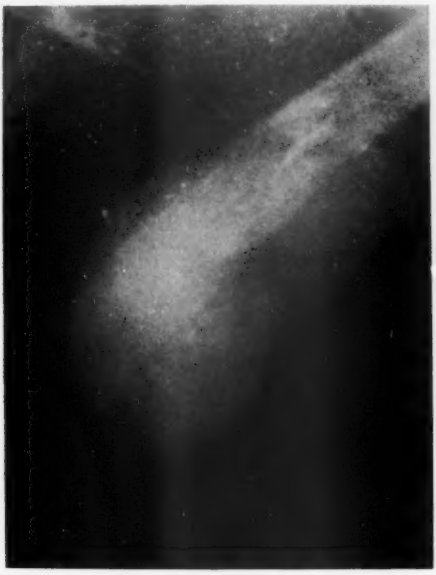


Fig. 3.—Cholecystogram made June 18, 1957, shows no change in the appearance of the fundal shadow.

At operation, on Oct. 22, 1957, a small, firm, fixed mass could be felt in the fundus of the gallbladder. The gallbladder was removed.

The resected organ contained no stones, but there was a mass, 1.6×0.8×0.8 cm. (Fig. 4), attached by a filamentous pedicle only 1 mm. wide. The tumor had a mammillated, nonulcerated surface. On section minute cystic spaces could be seen.

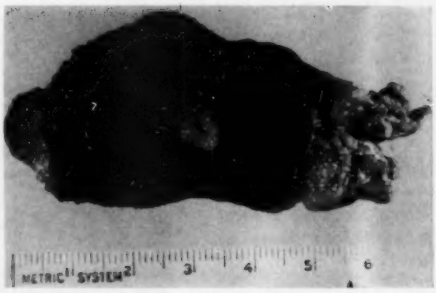


Fig. 4.—Resected gallbladder shows a firm polypoid tumor, 16×8×8 mm.

Microscopically, the wall of the gallbladder showed prominent mucosal folds with associated Aschoff-Rokitansky sinuses and cellular infiltration, indicating chronic inflammation. The polypoid structure was covered by a single row of columnar epithelial cells, similar to those of the mucosa of the gallbladder. The inner structure was composed of abundant loose, edematous connective tissue and a few scattered glandular structures, some of which were cystically dilated, with a mucosal lining similar to surface epithelium. The stroma was unusually abundant and in places compressed the glandular structures into slit-like spaces (Figs. 5, 6).

The pathologic diagnoses were chronic cholecystitis, Aschoff-Rokitansky sinuses, and fibroadenoma of the gallbladder.

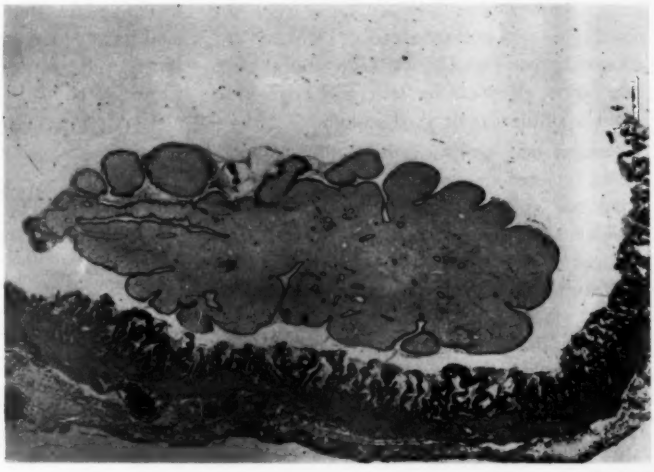


Fig. 5.—Low-power view of a section of the entire tumor as it lies on the mucosa. Notice the predominance of the connective tissue component and the lobulation of the surface. Hematoxylin-eosin stain; ×8.

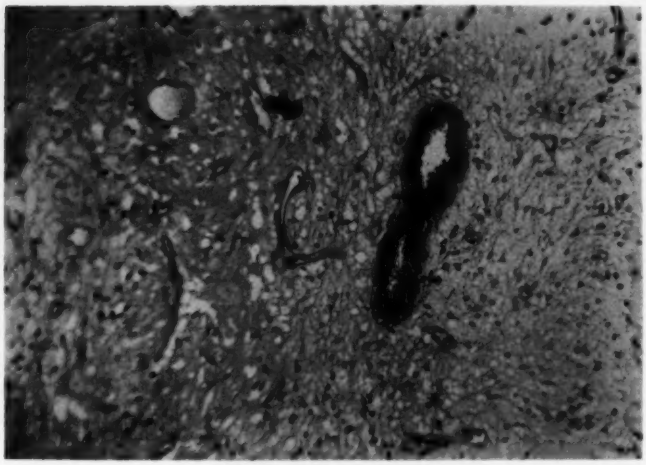


Fig. 6. — Photomicrograph showing detail of the fibroadenoma. The lesion is composed of edematous fibrous tissue and partially compressed glandular strictures. Hematoxylin-eosin stain; × 100.

On the eighth postoperative day the patient was discharged, after an uneventful convalescence. He has remained well.

Comment

Interest in benign neoplasms of the gallbladder has been greater in recent years because of the increasing frequency with which small tumors may be visualized by improved cholecystographic methods. We [1,2] previously presented a study of the benign mucosal tumors of the gallbladder encountered at the Ochsner Foundation Hospital. Papillary adenoma, nonpapillary (glandular) adenoma, and adenomyoma were the histologic types discovered. Two of the adenomas were associated with noninvasive carcinoma. Reports by others [3-7] have indicated a similar variety of lesions.

Benign neoplasms of the gallbladder are basically adenomas, since they are derived from the epithelial structures of the gallbladder. In this instance, the adenomatous element of the tumor is less impressive than the pronounced fibrous component in the stroma. This is in distinct contrast to the usual papillary or glandular type of adenomas, in which the fibrous tissue in the vascular stroma is limited. For this reason, we believe that this neoplasm can truly be labeled a fibroadenoma, comparable in all respects to the similarly classified tumors of the breast.

It is of further interest that this neoplasm had a truly filamentous stalk, which measured no more than 1 mm. in diameter. We previously described the fine pedicle that may attach cholecystic tumors to the wall of the gallbladder.

Summary

A case of fibroadenoma of the gallbladder is reported, apparently the first such lesion to be recorded. The neoplasm was visualized in cholecystograms during a period of seven years and had become slightly larger. It was successfully removed by cholecystectomy.

Ochsner Clinic, 3503 Prytania St.

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Age Changes in the Acetic Acid-Soluble Collagen in Human Skin

WILLIAM G. BANFIELD, M.D., Bethesda, Md.

Zachariadés,[1] in 1900, was the first to call attention to the fact that the age of the animal must be considered in any work dealing with the properties of collagen. Specifically, he intimated that age might affect the swelling properties of the substance. In 1927 Nageotte[2] found that after exposing the tail tendon of the rat to a low concentration of glacial acetic acid, the collagen that went into solution could be reconstituted as a precipitate by the addition of sodium chloride. Collagen also could be precipitated from the solution by neutralizing the acid or by dialysis. Using these methods, he was able to estimate the relative amount of collagen in acid solutions, to study the efficiency of various acids and acid concentrations in solubilizing the collagen and to compare the acid solubility of collagens from different sources. In 1934 Nageotte and Guyon[3] demonstrated for the first time an age difference in collagens. Collagen from tendons of young rabbits and young cattle could be extracted easily when exposed to dilute acids, but the collagen in the tendons of the old animals was completely, or almost completely, insoluble. A tendon from a 6-week-old infant gave a solution of collagen, whereas the authors were unable to obtain soluble collagen from the tendon of a man 70 years old. Tustanovskii[4] was able to obtain a soluble collagen from the skin of rats using an acid citrate buffer. Orekhovich[5] found that the amount of this collagen fraction was higher in the skin of young guinea pigs 2 to 6 weeks old than in animals over 7 months old. Recently Gross[6] has shown that the amount of sodium chloride-extractable collagen in the skin of guinea pigs greater than one year old is negligible when compared with that in young animals. In a previous publication, Banfield[7] demonstrated changes in the acetic acid solubility and swelling of human Achilles tendon with advancing age. In human skin,[7,8] acetic acid-soluble collagen was invariably present, usually in a high concentration, below one year of age. At one year of age there was a sharp reduction in this component of the skin collagen, and a further reduction in some persons over 30 years of age.

When citrate buffer or sodium chloride is used as the collagen-extracting solution, the method of extraction and the procedures for determining the amount of collagen in solution are complicated or time-consuming. This is likely to discourage the use of many tissues from which valuable information could be obtained concerning collagen if a simpler procedure were available. Such a method is now in use, employing dilute acetic acid as the extracting medium.[8] The procedure for estimating the amount of collagen in the acetic acid solution is also easy and rapid, and this was applied to the study of human abdominal skin, with the results to be reported here.

Methods

Human abdominal skin from the right upper quadrant was collected post mortem. Two-tenths gram, kept in a single piece as far as possible, was extracted in 5 cc. of 0.1% glacial acetic acid for 24 hours at about 9 C. About 1.5 cc. of the extract was then transferred to a 10×75 mm. test tube and about 0.3 gm. of sodium chloride crystals (analytical reagent, Mallenckrodt Chemical Works) added. If soluble collagen was present,

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Laboratory of Pathology, National Cancer Institute.

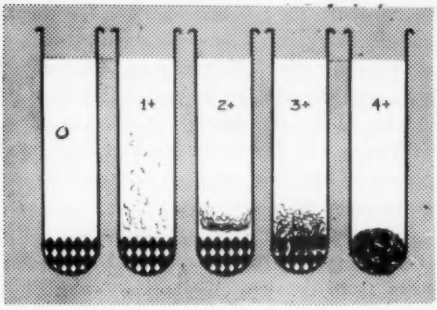

Fig. 1.—Diagrammatic representation of the grading of precipitates. Sodium chloride crystals are indicated as diamonds. A few strands of precipitate floating to the top of the tube is graded as 1+, a veil over the salt floating to the top, as 2+; a heavy precipitate sticking to the salt, as 3+, and a gel causing clumping of all the crystals, as 4+.

it precipitated in characteristic white fibrils, which were lighter than the extracting medium and tended to float to the top of the tube. The precipitate was graded visually and estimated at 0 to 4+. A few strands of precipitate floating to the top of the tube was graded 1+; a veil over the salt subsequently floating to the top, 2+, and a heavy precipitate sticking to the salt, 3+. A concentration of collagen in solution great enough to form a gel around the salt and causing all the granules to clump was read as 4+ (Fig. 1).

Hydroxyproline determinations were done, using the method of Wiss[9] on pooled samples of extracts representing precipitate readings from 0 to 4+ (Table).

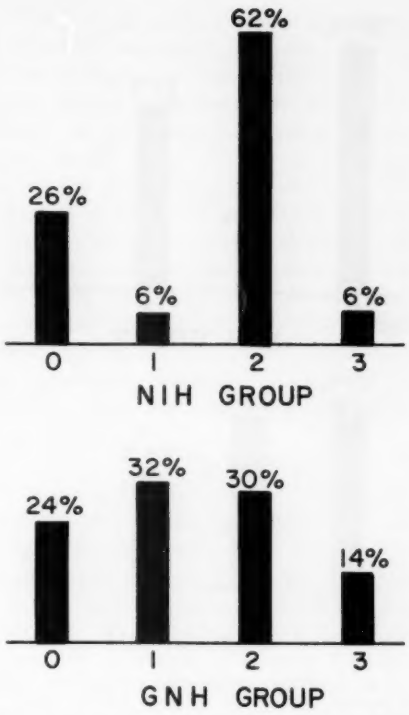

Fig. 2.—Acetic acid-soluble collagen in human abdominal skin from persons one to 30 years of age. Comparison of NIH with GNH group. Bars represent the percentage of subjects whose skin extracts yielded precipitates corresponding to the plus grading indicated at the bottom of the columns.

Results

Using the above method, the skin from 34 subjects between 1 and 30 years of age was tested at the National Institutes of Health (NIH) and compared with the results from 59 subjects of the same age group from the Grace-New Haven Community Hospital (GNH).

Figure 2 is a bar graph of the results for the NIH group and the GNH group. The bars represent the percentage of subjects whose skin extracts gave a precipitate of collagen corresponding to the plus reading at the bottom of each column. In both groups the percentage of subjects with no acetic acid-soluble collagen in their skin is essentially the same, between 24% and 26%. There is considerable variation, however, between the results from the two institutions in the percentage of persons falling within the 1+ to 3+ groups. The percentage of skin extracts with a 2+ precipitate is twice as high in the NIH group (62%) as in the GNH group (30%), and the number in the 1+ group is correspondingly lower for NIH and higher for GNH. This is probably the result of a difference in interpretation of the readings or a difference in the size of the salt crystals used for precipitation by the two observers.

In Figure 3 the results from skin extracts of subjects more than 30 years of age are presented in the same way—109 in the NIH group and 113 in the GNH group. There are 11% more subjects in the NIH group in whom no collagen could be ex-

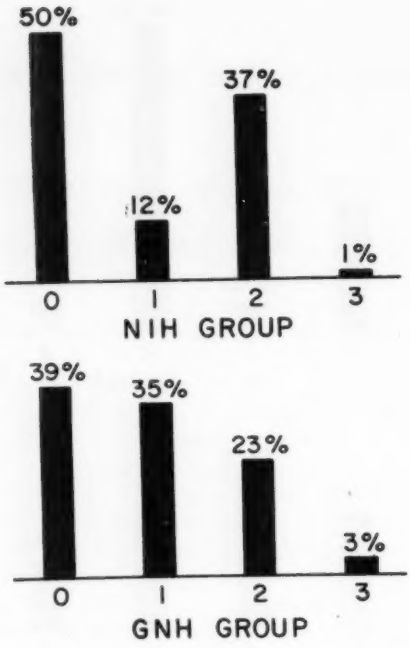

Fig. 3.—Acetic acid-soluble collagen in human abdominal skin from subjects greater than 30 years of age. Comparison of NIH with GNH group.

tracted from the skin than in the GNH group. This may represent a real difference between the two groups, but it would probably be impossible to analyze the difference with the data available. It is significant, however, in comparing Figures 2 and 3, that in both the GNH and the NIH observations there is an increase in the number of persons over 30 years of age whose skin collagen will not go into solution. The results, then, are similar in showing that a decrease in the acetic acid-soluble component of the skin collagen is found in the group over 30 years of age.

Though the finer plus readings of the two observers are not comparable, the results from the two institutions were sufficiently similar to warrant combining them if the results are divided into only two classes, consisting of a group in which skin extracts gave a precipitate and a group in which there was no precipitate. When this was done, the age groups could be broken up into 10-year periods, with the results shown in Figure 4.

The acetic acid-soluble collagen of the skin gradually disappears in 28% of persons by the age of 20 years, with a sharp decrease in the percentage of positive skin

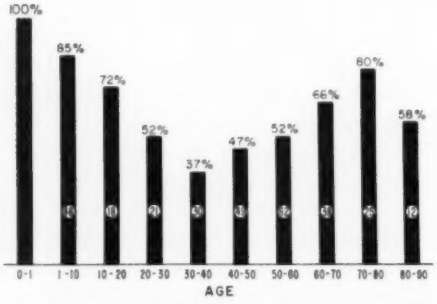

Fig. 4.—Age variations in acetic acid-soluble collagen in human abdominal skin. Bars represent the percentage of subjects in whom 0.2 gm. of skin extracted in 5 cc. of 0.1% glacial acetic acid for 24 hours yielded soluble collagen. The number of subjects tested is shown within each column.

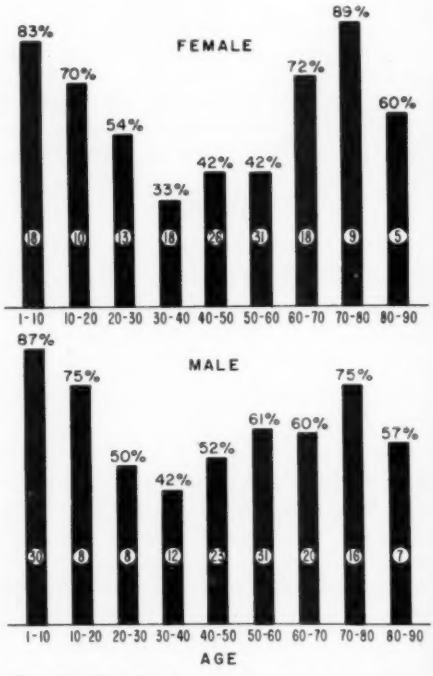

Fig. 5.—The data in Figure 4 have been broken down for comparison of males and females.

extracts between the ages of 20 and 30, and continuing until the age of 40. There is a rise between the ages of 40 and 50 and a continued rise to a high of 80% between the ages of 60 and 80 years, followed by a decline.

Figure 5 is a comparison of males and females. The graphs for the sexes are similar, and each corresponds to the graph for the combined results in Figure 4. There is a higher percentage of positive skin extracts in the younger and older age groups than in the intermediate age groups and a decline in the percentage after 80 years of age. There is a pronounced rise in the percentage of positive skin extracts from women between the ages of 60 and 70, whereas the rise for the men is more gradual. However, there must be a greater number of observations before sex differences will have statistical significance.

Several individual extracts giving similar readings were pooled for the hydroxyproline determination, and the results are presented in the accompanying Table.* As the amount of precipitate increases, the hydroxyproline content increases, indicating that the plus system truly reflects the relative amount of soluble collagen in the extract. In fact, a minimum visual precipitate detects the presence of dissolved collagen even below the sensitivity of the chemical method, which is 1μg. of hydroxyproline per cubic centimeter.

Comment

Though two observers seemed to differ in their interpretation of a precipitate as 2+ or 1+, the over-all results of the two studies were essentially the same. That is, in both instances there was a shift toward a lower concentration of acetic acid-soluble collagen in the skin of the group of persons over 30 years of age. However, if the criteria for grading the precipitate diagrammed in Figure 1 are strictly adhered to and the grain size of the sodium chloride is constant, the individual readings of different observers should be about the same. Readings should not be taken after the first minute or so, since in some instances a late opalescent precipitate forms, clearly different from the white, fibrillar collagenous precipitate.

It is clear from Figure 4 that there is a continuous orderly change in the amount of acetic acid-soluble collagen in the skin throughout life. Part of this change may be the result of a changing hormone pattern, reflected in the collagen metabolism. That this is possible has already been shown by injections of growth hormone into old hamsters, which resulted in an increase in the amount of acetic acid-soluble collagen in the skin. The actual change which takes place in the collagen fibril or collagenous composition of the skin in order to vary extractability, however, is not clear. It is probably the result of a combination of factors, such as variations in the rate of collagen formation, altering the amount of young, soluble collagen to old, insoluble collagen, maturation of young collagen, structural alteration in old collagen, or even collagen breakdown.

*I wish to thank Dr. Filadelfo Irreverre for these determinations.

Hydroxyproline Determinations of Soluble Collagen

Precipitate	Hydroxyproline, μg/Cc.	Collagen, μg/Cc.
0	<1	--
1+	<1	--
2+	1.6	11.4
3+	4.1	29.3
4+	11.4	81.4

Summary

The acetic acid-soluble collagen in human abdominal skin varies in an orderly fashion throughout life. Using 0.1% glacial acetic acid for 24 hours as the extracting medium, the soluble collagen gradually disappears from the skin in 28% of persons by the time they are 20 years of age. There is a sharp decrease in the percentage of positive skin extracts between the ages of 20 and 30 years, continuing until 40. After this

there is a gradual rise, reaching 80% between the ages of 60 and 80, followed by a decline.

I wish to thank Darlene Brindley for help in obtaining the data from the National Institutes of Health and Dr. Orlando Gabrielle for assistance in obtaining the data from the Grace-New Haven Community Hospital.

Laboratory of Pathology, National Cancer Institute.

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Experimental Production of Atheromatous Embolization

L. B. OTKEN Jr., M.D., Memphis

Embolism arising from ulceration of atheromatous plaques was first mentioned in the American literature by Benson in 1926, it being merely stated that such embolization could occur.[1] Since that time there has been a total of 41 documented cases. The first accurate account of the phenomenon was reported by Flory in 1945.[2] His nine cases demonstrated emboli in the spleen, pancreas, kidneys, and thyroid. Atheromatous embolization occurs predominantly in males with severe aortic atherosclerosis.

The significance of atheromatous emboli is questionable. In most cases the representative lesions are incidental findings. However, in reporting seven cases, Handler suggested that atheromatous embolization in the kidneys may cause or increase renal hypertension.[3] Probstein et al. reported 23 cases, in 12 of which emboli were demonstrated in the pancreas.[4] Of these 12 cases, 10 had an associated acute hemorrhagic pancreatitis. The authors concluded, therefore, that atheromatous embolization was an etiologic factor in acute pancreatitis.

The pathogenesis of these emboli is also questionable. Flory injected human atheromatous material into the ear vein of two rabbits and killed the animals one and seven days later, respectively.[2] With the first rabbit he demonstrated pulmonary emboli associated with panarteritis, and with the second, foreign-body giant cells and hyperplastic intimal tissue surrounding cholesterol crystals. The latter is the typical picture demonstrated by all authors reporting on this phenomenon. Zak and Elias were the first to suggest, from their observations on human materials, that the early stages of atheromatous embolization may manifest as a panarteritis with many eosinophils.[5] The literature would therefore suggest that atheromatous emboli are initially associated with panarteritis and later with a foreign-body reaction. However, Schornagel, in a recent report from the Netherlands, concluded from his observations that the appearance of foreign-body giant cells characteristically occurs early with cholesterol emboli.[6] Later the giant cells reportedly disappear and the crystals become enveloped by connective tissue.

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Memphis Section, National Heart Institute, and Institute of Pathology, University of Tennessee.

Observations

Three cases in which atheromatous embolization was an incidental finding were recently seen at this Institute. In all three instances there was severe, generalized atherosclerosis, with ulceration of numerous plaques in the aorta. The cause of death was myocardial infarction in the first two cases and carcinomatosis from carcinoma of the prostate in the third. There was nothing, either clinical or pathological, which could definitely be related to the finding of atheromatous embolization. Emboli were seen in the spleen, pancreas, kidneys, small intestine, and adrenals in the first case, in the spleen only in the second, and in the spleen and kidneys in the third.

The small arteries showing the emboli were characterized microscopically by slit-like spaces surrounded by fibrous tissue, which had filled the lumen partially or totally. Occasionally foreign-body giant cells were seen adjacent to the slits (Fig. 1), which contained a substance readily identi-

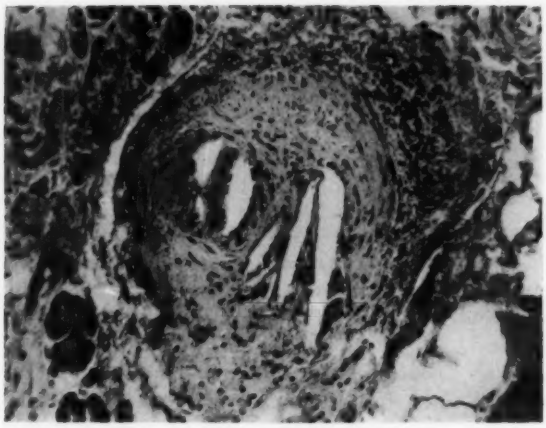


Fig. 1 (Human Case 1).—Atheromatous embolus in pancreas. Note foreign-body giant cell adjacent to crystal on right. Hematoxylin and eosin stain; × 200.

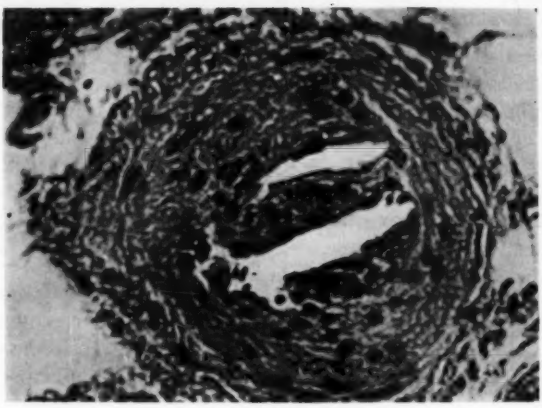
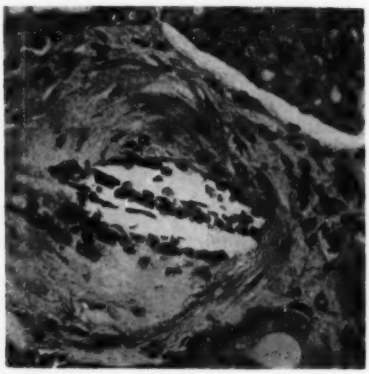


Fig. 2 (Human Case 1).—Atheromatous embolus in periadrenal arteriole. Hematoxylin and eosin stain; × 400.

Fig. 3 (Human Case 3).—Atheromatous embolus in kidney with blood in the acicular spaces. Hematoxylin and eosin stain; × 200.



fied as cholesterol, using polarized light on frozen sections. The crystals were sometimes seen protruding into the media of the vessels. Scattered mononuclear cells were seen around some of these arteries. In the third case, some arteries were seen to have the characteristic acicular spaces, but the spaces were seen to contain blood cells (Fig. 3). The last observation suggested that the cholesterol crystals disappeared in some fashion, leaving only an imprint in the vessel.

In order to study the pathogenesis of atheromatous embolization more fully, the following experiment was designed.

Materials and Methods

Atheromatous material was scraped from ulcerated atheromatous plaques of human aortas and suspended in saline. This suspension was shaken vigorously to hemolyze any red blood cells present, then centrifuged, and the atheromatous material resuspended in saline. The material was ground in a mortar until it would pass through an 18-gauge needle, the merosal N.F. (Merthiolate) added to a concentration of 1:10,000, and the suspension then stored at 4 C until used. Wetfield examination of this material revealed fibrin strands, nuclear debris, amorphous debris, and large numbers of variable-sized cholesterol crystals. Injection of 2.5 cc. of this suspension was made directly into the cardiac ventricular cavities of eight rabbits. Three received injections into the left ventricle, and the remaining animals, into the right. The rabbits were killed at intervals of one day to one month. One rabbit was injected with 2.5 cc. of pure cholesterol crystals suspended in saline and was killed on the following day.

Results

Two of the rabbits which were given injections into the left ventricle died immediately, autopsy revealing emboli in all three major coronary arteries. The third rabbit in which the left ventricular cavity was injected developed complete left-sided paralysis immediately after injection and died three days later, with classic signs of heart failure. Autopsy revealed emboli in the smaller coronary arteries with multiple small myocardial infarcts, and emboli in the brain, kidneys, spleen, and liver. Histological material from this animal was used to represent tissue reaction to the emboli three days after injection.

Reaction to the emboli 24 hours after injection was twofold. Emboli which were found to have mixed constituents, with or without cholesterol crystals, were associated with panarteritis (Fig. 4). The inflammatory cellular reaction consisted of neutrophilic and eosinophilic polymorphonuclear leukocytes. The second type of reaction was associated with small arteries in which single cholesterol crystals had lodged. These were manifest as cholesterol slits, surrounded by proliferating endothelial tissue (Fig. 5). Scant numbers of eosinophils were seen surrounding these arteries.

Reactions to the emboli three and five days following injection were essentially the same. A progressive organization by endothelial proliferation was associated with the mixed emboli. Eosinophils were still seen around the arteries. Single-cholesterol-crystal emboli became more difficult to find. The reaction to these was the same as that seen at one day except for formation of foreign-body giant cells adjacent to the crystals.

One month following injection there was almost complete organization of the mixed emboli by densely cellular fibrous tissue, but eosinophils were still present in abundance around the arteries (Fig. 6). Except

Fig. 4.—One-day-old experimental atheromatous embolus of mixed constituency. Hematoxylin and eosin stain; × 250.

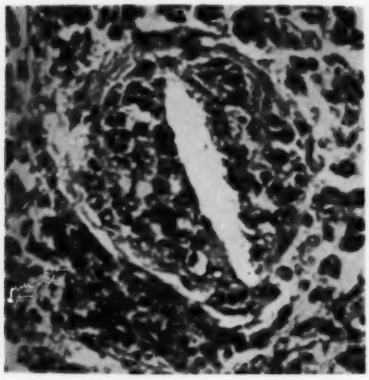


Fig. 5.—One-day-old single-cholesterol-crystal embolus. Hematoxylin and eosin stain; × 400.

for the eosinophils, these lesions had the appearance of ordinary organizing thrombi. No single-cholesterol-crystal emboli could be found after one month.

No evidence of the cholesterol crystals could be found in the rabbit into which pure cholesterol was injected, even after such a short interval as 24 hours.

Comment

It should be emphasized that there is a major difference between the experimentally induced lesions and the lesions most commonly seen in humans. Emboli consisting of mixed atheromatous elements were predominant in the rabbit. Such emboli are very rare in humans. No such lesions were seen in the three cases reported above. Should they occur, one would expect to see them associated with a panarteritis with many eosinophils.

Atheromatous emboli most commonly seen in humans are those consisting of single cholesterol crystals. The reaction to such emboli in the rabbit is a foreign-body type, which develops very rapidly. Time is apparently not an important factor in the development of the foreign-body reaction to cholesterol crystals, in contrast to Flory's [2] and Zak and Elias' [5] original impressions. The experimental results thus indicate that the type of emboli most commonly seen in humans, as demonstrated by Figures 1 and 2, probably represents very early lesions, rather than old ones.

It is considered important that with time the cholesterol-crystal emboli became more difficult to find in the rabbit, and finally could not be found at all. That suggests that the cholesterol is converted into a soluble state and subsequently leaves no evidence of the preexisting event. In any event, experimentally induced single-cholesterol-crystal emboli, of the type seen in man, apparently disappear rapidly. Arteries with slit lumina containing blood, as seen in Case 3 (Fig. 3), may represent the end-stages of this phenomenon.

Inability to find lesions in the rabbit into which pure cholesterol was injected was a surprise. The explanation is beyond the

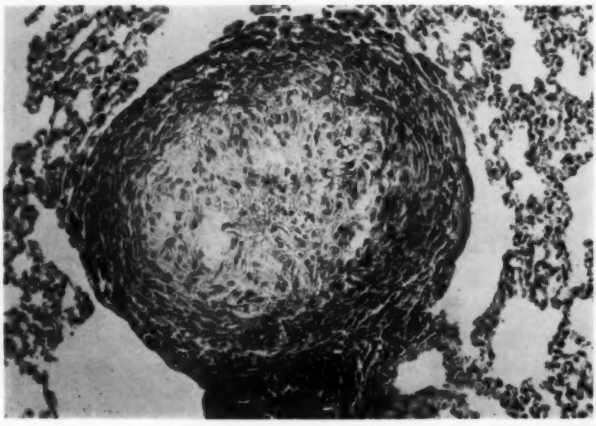


Fig. 6.—One-month-old experimental embolus of mixed constituency. Hematoxylin and eosin stain; × 200.

scope of this paper, but it is not felt to be a difference in particle size.

Summary and Conclusions

Atheromatous emboli were produced experimentally in rabbits by injecting human atheromatous material into the cardiac ventricular cavities. The animals were killed one day to one month after injection. Reactions to the emboli were twofold. Emboli of mixed constituency were associated with a panarteritis with many eosinophils, and these emboli organized very slowly. Single-cholesterol-crystal emboli were associated with a rapidly developing foreign-body reaction, becoming very difficult to find after only a few days' time, and finally could not be found at all. Three human cases are reported in which atheromatous embolization in various organs was an incidental finding. These cases are compared and contrasted with the experimental results.

In humans, ulceration of aortic atheromatous plaques occurs most commonly just proximal to the bifurcation. Should atheromatous embolization occur, therefore, the tissues most diligently studied at a routine autopsy would miss the lesions. However, cases in which there is ulceration of atheromatous plaques higher in the aorta are seen much more frequently than are atheromatous emboli. With this fact in mind, in conjunction with the experimental results of rapid disappearance of cholesterol-crystal emboli, it is suggested that atheromatous embolization occurs more commonly than is suspected but that the cholesterol crystals are rapidly converted into a soluble state and disappear. The emboli are apparently innocuous in the majority of instances.

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Renal-Cardiovascular Pathologic Changes in Aging Female Breeder Rats

GEORGE F. WILGRAM, M.D., and DWIGHT J. INGLE, Ph.D., Chicago

Female rats discarded as breeders by the Sprague-Dawley Company after casting six litters have at one year of age varying amounts of pathologic change.[1,2] A few animals are essentially normal, but most of them have some degree of renal-cardiovascular damage, and in a few it is dramatically severe. Some animals have hypertension; stomach ulcers occur rarely; mammary tumors develop in almost all animals during the second year of life, and malignant tumors are found in a few.

The pathogenic factors in these female rats have not been identified. Male rats of the same strain and age have relatively little disease. These rats have lived in an air-conditioned environment and were free from bacterial infections. Perhaps the very active reproductive life of the females is important. Diet is optimal for growth, reproduction, and lactation, but the possibility that diet affects the incidence and severity of the diseases associated with aging should be tested.

The pathogenic effects of hormonal overdosage in uninephrectomized rats on an abnormally high sodium chloride load are well known. From such studies it has been postulated that nonspecific forms of stress can, by derailment of the pituitary-adrenal cortex axis, cause disease.[3] Supporting evidence from experiments done under naturally occurring conditions is lacking so far. In the present studies we have tested the possibility that a severe stress or adrenal enucleation or stimulation of the adrenal cortices with corticotropin would, during a period of eight weeks, increase the incidence and severity of the diseases of aging female rats. One group of young adult male rats was subjected to neuromuscular stress. The results of each experiment were negative.

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The Ben May Laboratory for Cancer Research, University of Chicago.

Methods

Discarded breeder female rats 12 to 15 months old purchased from the Sprague-Dawley Company were fed Rockland rat diet ad libitum thereafter unless otherwise indicated. In Experiment 7, young adult male rats of approximately 300 gm. initial weight were used. Some groups of rats were fed a fluid medium carbohydrate diet (Table 1) either ad libitum or by stomach tube. In Experiment 2, 4% sodium chloride by dry weight was added to this diet. The rats were kept six per cage, at 73 to 78 F.

Grouping rats which have heterogeneous pathologic changes present at the beginning of the experiment is of critical importance. Controls and experimentals for any given experiment were always derived from a single shipment, it being known to us that different shipments are not uniform in respect to amounts and kinds of disease present. The animals were first arranged in order of weight and were numbered. They were then distributed into either two or three groups of ABBA or ABCCBA order, the assignment of

TABLE 1.—*Medium Carbohydrate Diet*

Constituent	Gm.
Cellu flour (Chicago Dietetic Supply House)	60
Osborne & Mendel salt mixture	40
Dried yeast (Pabst)	100
Wheat germ oil	10
Cod liver oil	10
Menadione (Vitamin K)	100 mg.
Mazola oil	200
Casein (Labco)	160
Stareh	200
Dextrin	190
Sucrose	200
Water to make total of	2,000 cc.

the heaviest rat to a group being a matter of chance. When weight is a variable, we have found this to be a satisfactory way to randomize unknown variables.

Depo-ACTH (Upjohn) and ACTHAR Gel (Armour) were the two repository forms of corticotropin given by subcutaneous injection in these experiments.

Severe neuromuscular stress was imposed by placing etherized animals on their backs on a board and securing them in that position for periods of 7 to 24 hours either once or twice each week. Upon awakening, the animals would struggle vigorously to release themselves. They appeared weak when released but had not injured themselves overtly during the stress.

To determine blood pressure at the end of the experiment, the rats were anesthetized with ether and the abdominal aorta was cannulated below the level of the renal arteries. The cannula was attached to a mercury manometer by a short length of fine-bore rubber tube filled with heparinized saline. The recorded pressure was that read when the anesthesia first became light enough to permit slight retraction of the rat's foot upon pinching the toes.

Following the determination of blood pressure, the rat was exsanguinated, autopsy performed, organs weighed, and gross pathologic changes noted in the heart, the aorta as it joins the heart, and the kidneys, mesenteric vessels (MA), and peripheral arteries (PA). Each rating of gross and of microscopic damage was on a scale of 0 (no damage) to 5 (severest).

At autopsy pieces of tissue were immediately placed in 10% neutral buffered formalin and allowed to fix for at least 48 hours. Selected pieces of tissue were placed in Bouin's fixative for 24 hours. The fixed material was prepared for paraffin sectioning, cut at 5μ, and stained routinely with hematoxylin and eosin. Sections of Bouin-fixed material were allowed to remain in 70% alcohol overnight to remove any excess picric acid prior to staining. Selected sections were stained with Lendrum's picric acid-Mallory method for detection of fibrin.[4] The adrenal glands were fixed in 10% neutral buffered formalin. Frozen sections were cut at 5μ and stained for fat with a 0.4% solution of oil red O in a mixture of 1:3 v/v triethylphosphate in distilled water.

The aorta was cut lengthwise. Each heart and kidney was sectioned at three different levels to allow for a better histological survey of the organ. Several mesenteric vessels were "bundled" and processed together and cut in cross section, so that each slide offered at least four or five mesenteric arteries for histological review.

Histological Grading of the Lesions

The grading system used in this study has certain shortcomings in that it assesses the morphology of the induced lesions only and does not take into account the fact that similar morphological changes result from different causes and pathological processes, or that qualitatively different pathologic processes may be represented by our different classes of damage. No final conclusions may be drawn by analysis of the lesions as they are presented. However, for the purpose of orientation and rough estimation, this system has proved useful in the past, and in this study it seemed justified. This grading system differs from all other grading systems used in previous studies reported from this laboratory.

Grading System

Heart:
- 0—Normal
- 1—Occasional lymphocytic focal infiltration in myocardium
- 2—More of 1
- 3—Widespread interstitial myocarditis
- 4—More of 3+ myocardial focal necrosis
- 5—More of 4

Blood Vessels
- 0—Normal
- 1—Slight edema of vessel wall with thickening and hypertrophy
- 2—Same as 1+ irregularities of luminal contour and occasional intimal protrusion
- 3—Marked intimal protrusions with bizarre luminal outline; rigidity of vessel wall, as illustrated by widening of lumen and thinning of vessel wall, which assumes frequently a ground-glass appearance
- 4—More of 3+ more marked signs of medial injury to vessel wall; occasional necrosis of media
- 5—Frank necrosis of media with calcification

Aorta
- 0—Normal
- 1—Slight changes in ground substance of media with irregularities in arrangement of elastic lamellae and smooth muscle fibers
- 2—More of 1
- 3—Disintegration and necrosis of elements of media
- 4—More of 3+ extensive calcification; "fractures" of calcified areas
- 5—More of 4+ intimal proliferation as sign of reaction to changes in underlying part of media; extensive "knob formation," leading to bamboo-stick appearance of aorta in the gross

TABLE 2.—*Pathology in Sprague-Dawley Female Rats Discarded as Breeders at About One Year of Age: Averages and Standard Errors*

Experiment	Body Wt., Gm.		Heart, Mg.	Kidney, Mg.	Thymus, Mg.	Adrenal, Mg. 1	HG BP	Pathology										
								Heart			Kidney		Pancreas		Arteries Aorta		Arteries Gross	
	Initial	Final						Muscle	Vessels	Gross	Paren.	Vessels	Paren.	Vessels	Gross	Micro.	MA	PA
1	Eat Chow diet ad libitum. Neuromuscular stress 7 hr 1 per week for 3 wk.; then 24 hr. 1 per week for 5 wk.																	
Controls	386	410	1,127	1,264	135	30	135			1.70								
N=18	±5.6	±9.2	±26	±33	±20	±1.0	±3.3			±0.39								
Stress	387	348	1,143	1,215	63	34	120			1.00								
N=16	±5.4	±3.8	±26	±23	±4.1	±1.0	±3.2			±0.21								
2	Eat 4% salt diet ad libitum. Neuromuscular stress 7 hr. 1 per week for 2 wk.; then 24 hr. 1 per week for 6 wk.																	
Controls	395	433	1,353	1,313	122	30	133			1.64								
N=11	±5.5	±12.3	±44	±47	±11	±0.9	±4.1			±0.36								
Stress	390	355	1,284	1,225	80	34	136			1.60								
N=10	±7.1	±8.6	±54	±41	±8.0	±1.3	±4.6			±0.26								
3	Tube-feed low-salt diet. Neuromuscular stress 6 hr. twice each week for 8 wk.; weigh both kidneys																	
Controls	398	476	1,269	2,378	135	33	129	0.19	1.42	0.62	2.17	1.47	0.08	0.87	0.37	0.73	0.46	1.80
N=12	±5.6	±6.8	±33	±48	±18	±1.1	±1.8		±0.16	±0.14	±0.22	±0.16		±0.1	±0.28	±0.19	±0.25	±0.22
Stress	406	445	1,347	2,448	89	46	126	0.29	1.68	0.71	2.33	1.77	0.33	0.96	0.54	1.08	0.33	1.37
N=12	±8.0	±7.0	±28	±81	±11	±1.1	±5.0	±0.08	±0.15	±0.31	±0.20	±0.17	±0.11	±0.08	±0.33	±0.24	±0.22	±0.36
4	Eat Chow diet ad libitum. One group, adrenal enucleation; one group long-acting ACTH (Upjohn) 1 unit per rat per day for 8 wk.																	
Controls	362	368	1,124	1,197	99	29	124	0.10	1.40	0.72	1.90	1.40	0.05	1.00	1.1		0.90	0.90
N=20	±6.7	±6.4	±22	±32	±5.5	±1.0	±7.1		±0.09	±0.09	±0.08	±0.06		±0.2	±0.19		±0.09	±0.09
ACTH	362	367	1,116	1,136	110	33	125	0.10	1.10	0.52	2.00	1.40	0.02	1.20	1.2		0.70	0.80
N=20	±6.2	±7.3	±23	±28		±0.9	±4.4		±0.1	±0.14	±0.2	±0.19		±0.2	±0.28		±0.2	±0.21
Enucleate	362	358	1,093	1,151	103	23	131	0.02	1.74	0.45	1.87	1.55	0.0	0.92	0.8		0.80	1.0
N=19	±7.8	±7.9	±17	±24	±7.1	±1.3	±2.3		±0.13	±0.02	±0.12	±0.15		±0.09	±0.09		±0.09	±0.1
4	Eat Chow diet ad libitum; 1 u. long-acting ACTH (Armour) 3 times per week for 8 wk.; weigh both kidneys																	
Controls	363	454	1,156	2,428	134	35	126	0.03	1.31	1.31	1.87	1.56			0.87	0.81	0.75	0.81
N=8	±4.4	±8.2	±23	±78	±10	±1.4	±3.3		±0.13	±0.36	±0.21	±0.12			±0.24	±0.30	±0.28	±0.09
ACTH	359	455	1,140	2,304	102	38	126	0.33	1.44	0.81	1.66	1.72			0.31	0.17	0.41	0.97
N=16	±2.6	±3.6	±18	±63	±10.5	±1.2	±2.3	±0.09	±0.14	±0.09	±0.15	±0.16			±0.09	±0.04	±0.10	±0.21
5	Eat Chow diet ad libitum; 2 u. long-acting ACTH (Upjohn) per rat per day for 8 wk.; weigh both kidneys																	
Controls	393	400	1,535	2,767	88	31	125	0.30	1.25	0.95	2.15	1.70	0.0	1.00	1.45	2.25	1.35	1.50
N=10	±3.9	±14.4	±24.5	±128	±8.5	±1.7	±4.1	±0.07	±0.12	±0.2	±0.19	±0.16		±0.1	±0.5	±0.4	±0.37	±0.22
ACTH	397	410	1,581	2,804	75	39	128	0.31	1.59	1.20	2.42	1.87	0.0	1.00	1.50	2.04	1.30	1.20
N=12	±4.2	±7.5	±93	±61	±7.3	±1.1	±4.0	±1.10	±0.16	±0.3	±0.25	±0.19		±0.2	±0.55	±0.5	±0.37	±0.22

6	Eat Chow diet ad libitum; 4 or 8 u. long-acting ACTH (Upjohn) per day for 8 wk.; weigh both kidneys															
Controls	356	359	1,093	2,261	90	31	124	0.33	1.58	0.87	1.33	1.58	1.33	1.10	0.79	1.04
N=12	±6.7	±6.1	±26.9	±60.4	±10	±0.88	±1.2	±0.12	±0.16	±0.2	±0.12	±0.16	±0.44	±0.2	±0.39	±0.23
ACTH (4 u)	354	354	1,103	2,804	58	41	126	0.60	1.52	0.92	1.21	1.50	0.79	0.96	0.92	1.04
N=12	±6.0	±7.1	±24.7	±74.5	±7.4	±1.4	±0.33	±0.1	±0.13	±0.17	±0.19	±0.16	±0.23	±0.3	±0.27	±0.23
ACTH (8 u)	359	352	1,114	2,306	67	64	131	0.29	1.39	0.87	1.54	1.79	0.75	0.75	0.58	0.62
N=12	±8.7	±17.2	±34.4	±64.9	±3.8	±1.8	±3.1	±0.06	±0.14	±0.22	±0.18	±0.16	±0.52	±0.2	±0.27	±0.22

Kidney

0—Normal

1—Occasional lymphocytic focal infiltrates, predominantly close to renal pelvis; slight glomerular enlargement and thickening of capillary loops

2—More of 1+ occasional hyaline eosinophilic casts; occasional widening of Bowman's space with shrinkage of glomerulus; incipient cloudy swelling and degeneration of convoluted tubules; early picture of what has been termed "obstructive nephropathy"

3—Lymphocytic and polymorphonuclear infiltrates common, frequently also in cortical areas; widespread cast formation with signs of cell proliferation and repair in convoluted tubules; glomerular changes range from edema of capillary loops to shrinkage and partial adhesion of glomerular loops to internal membrane of Bowman's capsule; fibrinous exudates within space of Bowman are common

4—More of 3; inflammatory and proliferative changes of glomerular capillary loops are pronounced; necrosis of glomerulus with following hyalinization commonly observed; cast formation extensive; degeneration of convoluted tubules advanced; afferent glomerular arterioles either hyperplastic, showing signs of inflammatory infiltration, or beginning to exhibit signs of arteriolar necrosis

5—More of 4; histological picture is indistinguishable from a full-blown picture of severe nephrosclerosis

Experiments and Results

The information on experimental conditions, numbers of rats, and results from Experiments 1 to 6 are in Table 2. The varying degrees of pathologic change observed are described below. There were no established differences in the incidence and severity of disease between any two groups in any experiment, although there were differences from one experiment to another when observations were made at different times and upon different shipments of rats from the Sprague-Dawley farms.

In Experiment 7 (Fig. 1), blood pressures were determined on 180 young adult male rats after periods of neuromuscular stress for 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 720 minutes. After four hours of neuromuscular stress, dark-red adrenals were a regular finding, and hemorrhage

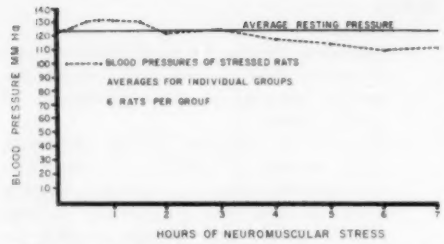

Fig. 1.—Average blood pressures of groups of young male rats after different periods of neuromuscular stress.

into the thymus was occasionally seen. One rat under stress for three hours had three bleeding "ulcers" in the stomach; two other animals had blood in the stomach, and one other had blood in the lower intestine, but the sites of hemorrhage could not be found. Hemorrhages within the mesentery were observed twice. The average blood pressure was somewhat elevated at periods of 30-90 minutes and thereafter declined to lower than normal values (Fig. 1).

Weight.—There was a tendency for the control rats of the adult series and those treated with small doses of corticotropin to gain some weight. Rats given 4 and 8 units of corticotropin daily, and most of the rats subjected to neuromuscular stress, showed some loss of weight.

Fig. 2.—Myocardial lesion in old female breeder rat. Connective tissue and a focal lymphocytic infiltrate have replaced the most superficial part of the myocardium. Low power; H.&E. stain.

Blood Pressure.—Among young adult rats, the average mean pressure as determined by our procedure is 122 mm., and a blood pressure above 140 mm. is rare. All but one average value in this series was somewhat higher than the normal average. Of a total of 220 old female rats, 42 had pressures of 140 mm. or above, the highest being 174 mm. Hypertension was not related to any of the experimental procedures but occurred with equal frequency among control groups.

Heart.—Cardiac hypertrophy can be caused by hormonal overdosage in salt-loaded rats and is commonly observed in animals with hypertension. Average weights of hearts were not significantly affected by the procedures tested here. No visible lesions were seen in any heart in this series of experiments, although Ingle and Baker [1] have observed white spots representing necrosis in the hearts of a few discarded female breeders of this same strain.

The cardiac muscle of some rats exhibited focal lymphocytic infiltrates surrounded by mild edema. Very infrequently, small focal fibrotic areas could be dis-

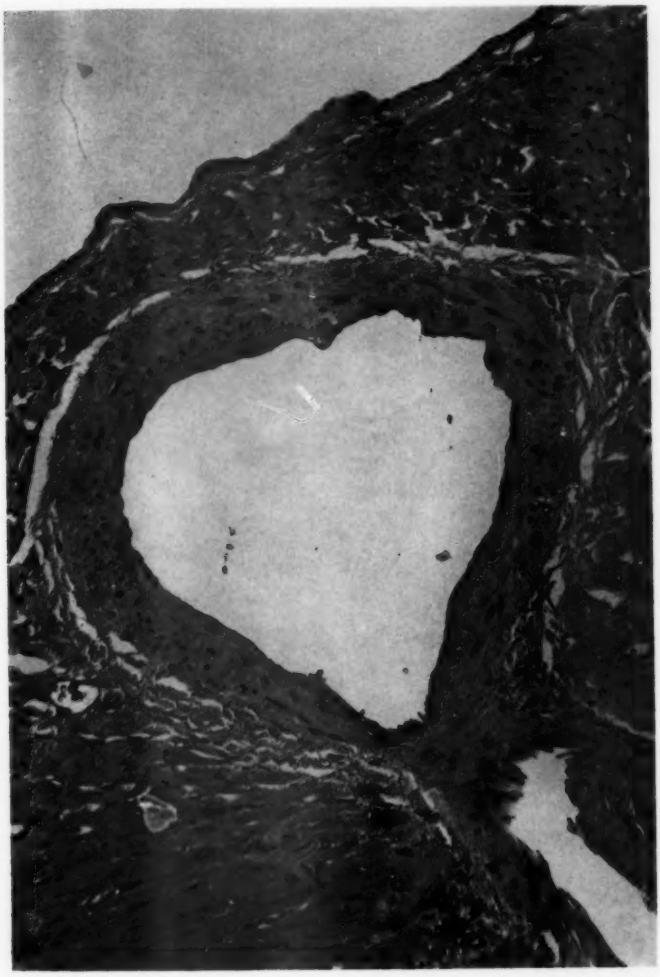

Fig. 3.—Coronary lesion of the mildest degree. The arterial wall is slightly thickened and edematous. There is some irregularity of the endothelial contour. A spur-like, tiny endothelial protrusion, consisting mainly of an increased amount of connective tissue ground substance, is visible in the upper part of this coronary artery. Low power; H.&E. stain.

cerned, but no cardiac infarcts were detected. The cardiac valves of some rats showed fibrous nodules and an increase in the subendocardial connective tissue ground substance. The coronary arteries were subject to a variety of changes of a kind which has been described by other workers.[1,5-7] Most commonly the wall of the coronary arteries appeared thickened and slightly edematous. The muscular layer was frequently hyperplastic; the intima showed irregular foldings, with small nodules protruding into the lumen. These protrusions consisted of a slightly increased number of endothelial cells "sitting" on nodules which represented an increase of subendothelial connective tissue ground substance. Less frequently the media showed signs of calcification and condensation. When the latter occurred, an irregularity of the vascular contour was always strikingly noticeable. The vascular lumen in these cases was widened.

Kidney.—Hormonal overdosage, which tends to cause renal-cardiovascular diseases in the rat, causes the kidneys to become heavier. Significant changes in the weights of kidneys were not produced by any of

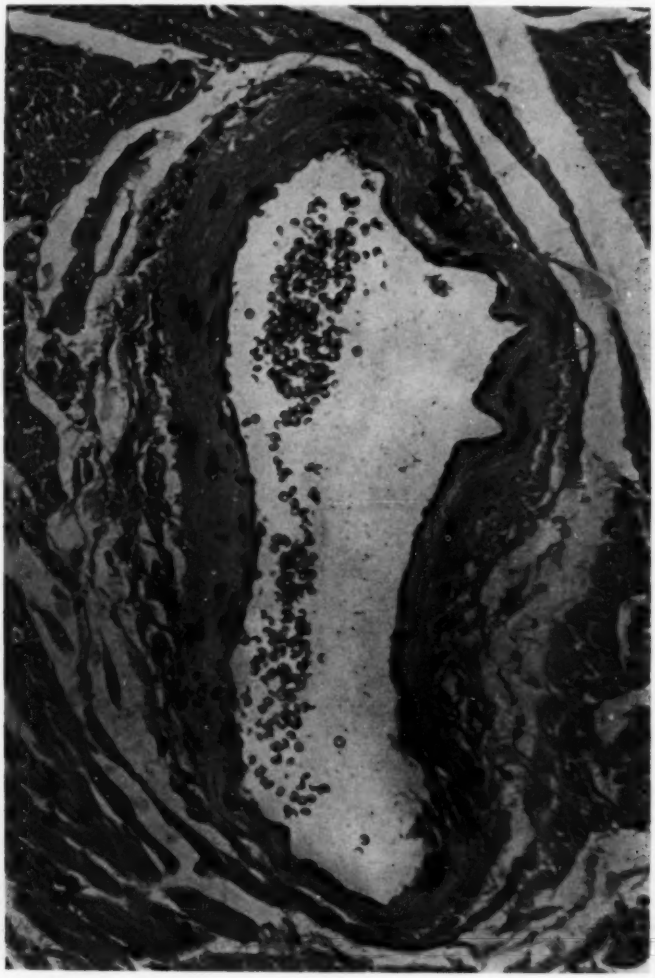

Fig. 4.—More advanced coronary change. The endothelial contour is distinctly irregular and bizarre. The media of the arterial wall is partly thinned and rigid. The overlying intima is heaped up into protrusions. Serial sections and similar lesions observed in the same animal in kidney, pancreas and mesentery rule out artifacts. Medium power; H.&E. stain.

the procedures tested in these experiments. Almost all kidneys showed some degree of gross change. Minor changes included a few tiny pits and nodules, the latter being due to hypertrophied tubules. Other kidneys had varying degrees of roughness; some had yellow and gray spots, representing inflammation and necrosis, and the few most severely damaged were pale, uniformly yellow, and very nodular.

The vascular lesions in the renal arteries were identical with or comparable to those observed in the coronary arteries. The parenchymatous changes in the kidneys resembled closely the ones that have been described as "obstructive nephropathy."[8-10] Small areas of focal lymphocytic infiltrates were clustered most commonly around the renal pelvis. Hyaline, eosinophilic casts were seen in varying frequency and size, obstructing mostly the collecting tubules but reaching not infrequently up to the cortex. The glomeruli belonging to these obstructed tubules showed changes that varied from a thickened glomerular membrane over edema of the capillary loops to shrinkage of the whole glomerulus, with an increase in width of Bowman's space. Occasionally the process had progressed to hyalinization and complete obliteration of the whole glomer-

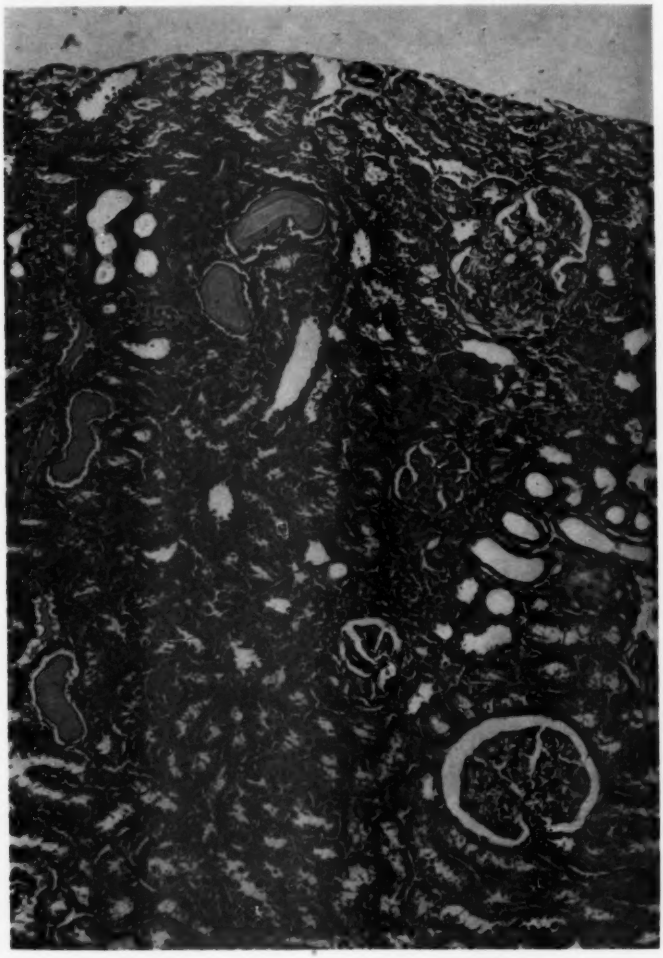

Fig. 5. — Average degree of obstructive nephropathy as observed in the aging female breeder rat. The glomerular changes range from shrinkage of the capillary loops to adhesions and in part to obliteration. Hyaline, colloid casts are the most conspicuous change in the tubular apparatus. These casts are most commonly found in the collecting tubules, but frequently reach as high as the distal convoluted tubules. Both proximal and distal convoluted tubules exhibit signs of parenchymatous degeneration. Areas where the tubular epithelium is flattened and the tubular lumen widened are commonly observed. Low power; H.&E. stain.

ular apparatus, but these severe changes were rare in this series of animals. Either hypertrophic convoluted tubules or convoluted tubules greatly extended by the obstruction lower down protruded as nodules above the surface of the neighboring kidney surface, while scar formation in the glomeruli and necrosis of the adjacent convoluted tubules tended to retract the surface of the kidney. The convoluted tubules very commonly showed signs of fatty and parenchymatous degeneration, leading sometimes to necrosis and complete disappearance of the normal architecture of the renal cortex.

Thymus.—Weights of thymus in these aging rats were all below the range of values found for young adult animals; stress and corticotropin each caused further atrophy of thymus.

Adrenal.—Stress caused some increase in weight of adrenals, and corticotropin caused an increase in adrenal weights that was correlated with dosage. Histologically this was borne out by an increase in width of the fasciculate zone, which, when stimulated by higher doses of corticotropin, stained somewhat less intensely for fat than that in the controls. Our fat stains revealed

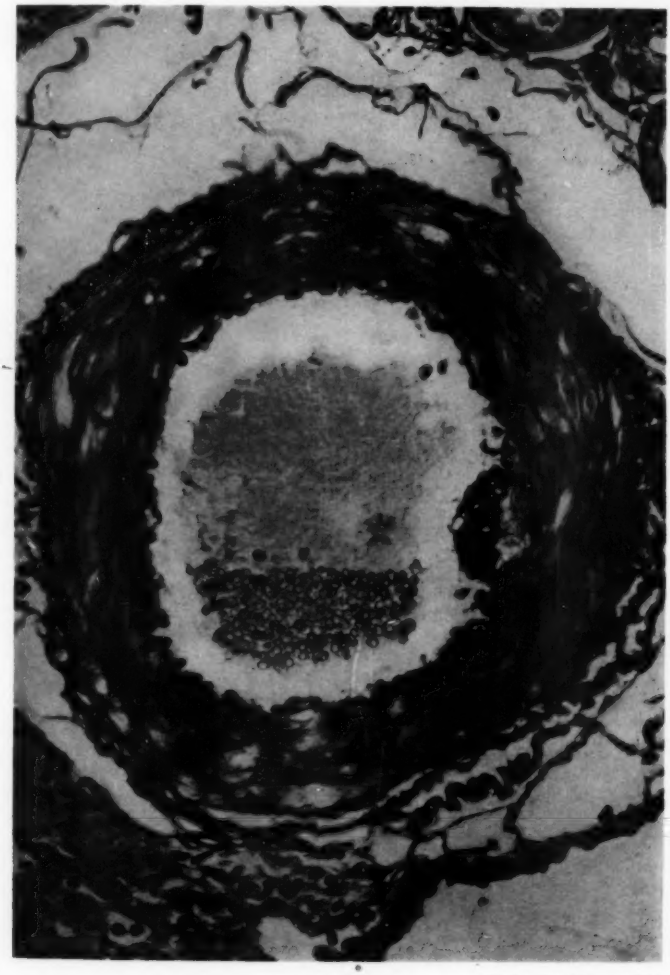

Fig. 6.—Earliest change in renal artery. The vessel wall appears slightly thickened and edematous. The internal elastic membrane of this renal artery is coiled up and protrudes as a "knob" into the lumen. There is a slight change in the tinctorial characteristic of the underlying connective tissue ground substance. High power; Lendrum's picric acid-Mallory method.

no particular changes in the zona glomerulosa.

Mesenteric and Pancreatic Vessels.— Varying degrees of inflammatory changes in arteries cause visible lesions, especially in the vessels of the mesentery. The arteries can show slight to marked kinking and spiraling, with varying degrees of swelling and hardening, changes which, in their severest form, appear as grape-like clusters of dark nodules throughout the mesenteric vessels, and rarely in the pancreatic vessels. The nodules closely resemble panarteritis nodosa. The ratings of damage to the mesenteric arteries are given under MA in Table 2.

Changes similar to those of the coronary and renal arteries occurred histologically in the vessels of the pancreas and mesentery, except that protrusion into the lumen of the vessels of these latter organs was usually more pronounced than in the coronary arteries, while medial hypertrophy was commonly less conspicuous. In isolated cases, inflammatory, mostly lymphocytic, infiltrates could be seen invading all layers of either mesenteric or pancreatic arteries. These changes are most favorably compared with panarteritis-nodosa-like lesions, as defined in human conditions, although direct comparisons may not be permissible.

Fig. 7. — Advanced changes in a main stem of the renal artery of an old female breeder rat. Bizarre protrusions of the intima caused by advanced changes in the media give the lumen of this vessel a strikingly irregular contour. The lesions in the media consist of changes in the ground substance, leading partly to thinning and partly to thickening of the vessel wall, which is edematous. Judging from a vacuole to be seen in the right upper part of the distorted vessel, there is apparently also fat accumulating in some areas of the injured vessel wall. Medium power; Lendrum's picric acid-Mallory method.

Aorta and Peripheral Arteries.—The thoracic aorta showed varying degrees of rigidity and change from its normal appearance as a blue elastic vessel. It became rough and rigid, with a patchy whiteness somewhat resembling trachea. This rigidity sometimes extended throughout the entire arterial tree. Ratings were made of the degree of rigidity and kinking seen in the femoral artery of the leg. (TA in Table 2 indicates thoracic aorta, and PA, peripheral artery.)

Microscopic examination of the aorta only rarely disclosed intimal changes. These took the form of irregularities in the otherwise even contour of the aortic intima. The irregularities were caused by a slight increase in endothelial cells, superimposed on a small increase in connective tissue ground substance. The internal elastic lamella was neither frayed nor interrupted. All the more striking changes occurred in the media. Here splitting of the elastic membranes, disintegration of the muscular layers, and replacement of them by necrosis and calcified debris produced a picture that resembles closely the one designated as "Mönckeberg's medial sclerosis" in the hu-

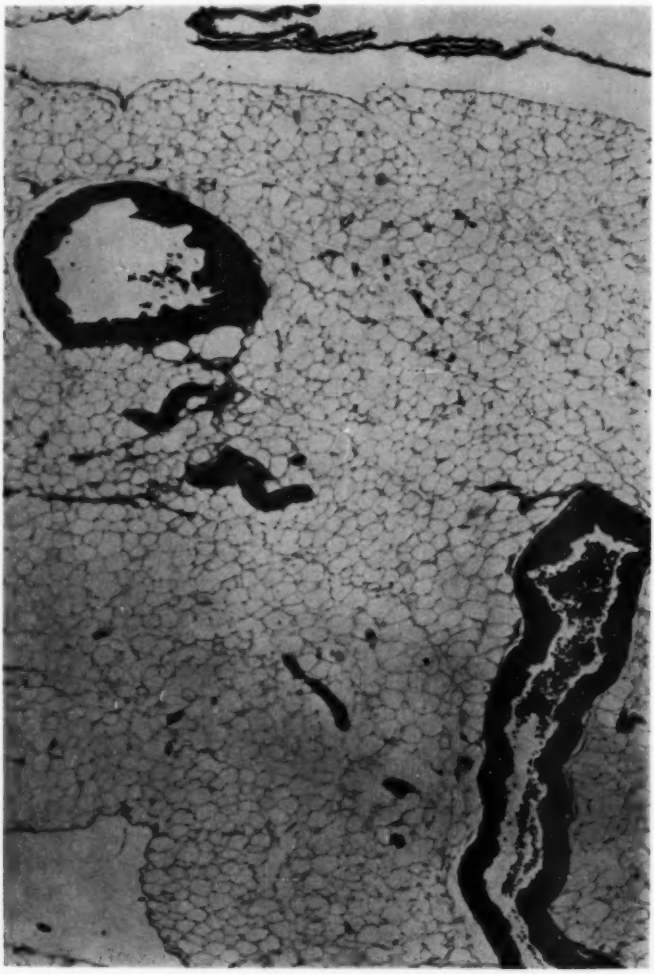

Fig. 8.—The mesenteric arteries of the animal whose coronary arteries are shown in Figure 4 exhibit marked kinking and thickening of the vessel wall. The media shows great variations in thickness. The overlying intima is heaped up in bizarre protrusions, consisting mainly of increased amounts of connective tissue ground substance.

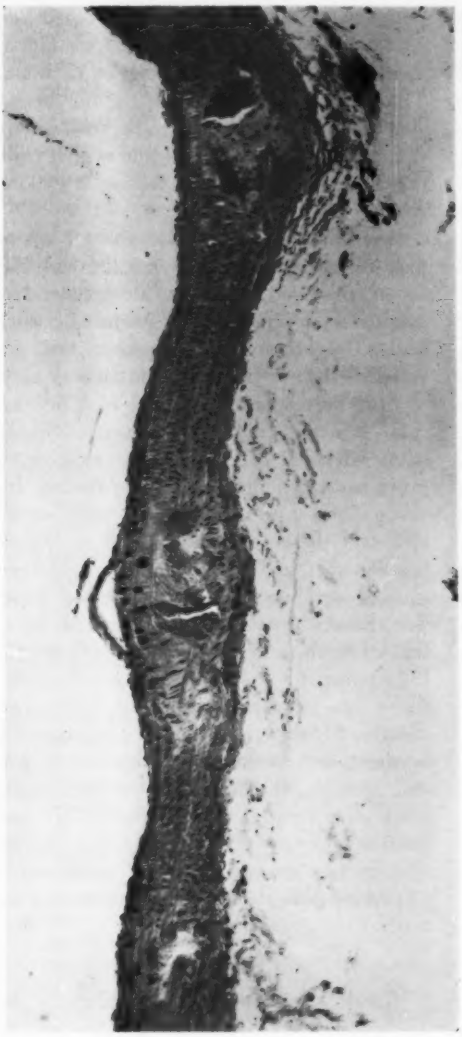

Fig. 9.—Medial sclerosis of the aorta. The normal structure of the aorta is interrupted in three places in this photograph of an aorta, showing a typical bamboo-stick appearance in the gross. Necrosis, calcification, and "knob formation" are pronounced. Lowest power; H.&E. stain.

man subject. The necrotic calcified material sometimes bulged toward both the interior and the exterior of the aortic wall, forming knob-like protrusions on both sides. These were responsible for the bamboo-stick-like appearance of the aorta in the gross.

Tumors.—All the rats were ostensibly free from tumors when the experiments were started. Eight rats developed mammary fibromas during the two months of observation. One had a fibroma of the uterus. There was no apparent relationship between the appearance of tumors and the treatment the rat received. No other tumors were detected.

Other Changes.—Each of three rats was found to have a stomach ulcer, and one rat had a cystic kidney.

Comment

Although the discarded breeder female rats have diseases which do not closely simulate the common degenerative diseases of man, it would be interesting to know

the causes of these diseases. These diseases are not aggravated by severe neuromuscular stress during a period of two months. We tested the possibility that this form of stress might cause either acute myocardial infarction or cardiac necrosis in these rats, some of which have a severely diseased vascular tree. This is the form of stress found by Selye [11] to cause cardiac necrosis, but not infarction in rats given large doses of certain sodium salts plus doses of sodium-retaining steroids.

The reasons for our negative findings on the effect of corticotropin in these old female rats, as opposed to the positive results claimed by Wexler and Miller,[12] are not known to us. Although uninephrectomy was done in some of their rats, they state that it is not necessary for the production of arteriosclerosis. The details of their procedures and data on frequency and extent of pathologic change in control animals and in rats given corticotropin have not been published.

The pathogenesis of the vascular lesions seen in these aging female breeder rats is not known. We are inclined to believe that the diseased kidney, and electrolyte disturbances ensuing from renal malfunction, among other etiological factors, play an important part in the development of these vascular changes. It is conceivable that the numerous pregnancies of these female rats promote ascending pyelonephritis and obstructive nephropathy. Once the kidney is damaged, hypertension and vascular injury may follow. This notion is strengthened by the fact that a variety of experimental procedures that produce kidney damage of one kind or another are followed by a predominantly medial type of vascular abnormality in the rat. These experimental procedures range from production of hypervitaminosis D [13-15] and choline deficiency,[16-18] sulfathiozole precipitation in the tubules,[19] parathyroid excess,[20] and injection of anti-rat-kidney serum [21] to the Goldblatt type of reduction or total interruption of renal blood flow.[22,23] While all these experimental procedures are decidedly different and lead to various forms of kidney damage, they have one thing in common: They lead to a reduction of the number of functioning nephrons and are followed in the rat by vascular lesions, the medial component of which exhibits the most striking change.

We, therefore, speculate that a major part of the pathogenesis of the vascular lesions observed in our female breeder rats originates in frequent pregnancies, which lead to obstructive nephropathy; this, together with electrolyte disturbances, may, in turn, be responsible for the ensuing vascular injury. While this speculation leaves many detailed questions unanswered, it gives us a hypothesis to guide further investigations. Our data do not support the idea that these vascular changes in female breeder rats are due to nonspecific stress, causing derailment of adrenocortical function. Severe neuromuscular stress or injection of small to large doses of corticotropin to activate the adrenal cortices does not cause an increase in damage after two months of experimentation.* This period is short, but overdosage of rats with salt and steroids is claimed to imitate conditions of stress causing hypertension and renal-cardiovascular pathologic change within four weeks. Further experiments employing longer periods of stress are in order.

Summary

Female rats discarded as breeders by the Sprague-Dawley Company at the age of one year have varying amounts of renal-cardiovascular pathologic change. Some animals have stomach ulcers; some have hypertension; benign tumors are common, and malignant tumors occur in a few. Treatment of these rats with varying doses of repository forms of corticotropin (Depo-ACTH and ACTHAR Gel) or repeated exposure of rats to severe neuromuscular stress for periods of eight weeks failed to

* D. Lehr and associates have reported results very similar to ours.[24]

produce a significant change in the incidence and severity of pathologic change over that observed in control groups. It is felt the chronic obstructive nephropathy and ensuing electrolyte disturbances are probably important factors in the cardiovascular pathology observed in these aging female breeder rats.

The Ben May Laboratory for Cancer Research, The University of Chicago (Dr. Ingle).

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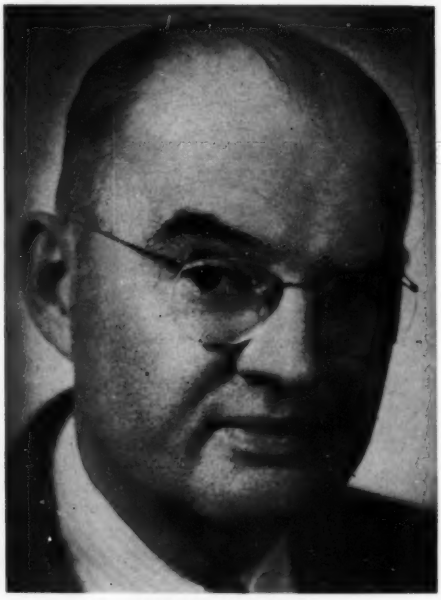
Obituaries

JAMES B. McNAUGHT, M.D.
1894-1959

Dr. James B. McNaught, long-time great teacher in pathology at Stanford University School of Medicine and at the University of Colorado School of Medicine, died of coronary occlusion and cardiac infarction at the Veterans Administration Hospital, Albuquerque, N. Mex., on Aug. 7, 1959. He sustained this serious lesion in the early morning of Aug. 5, while on an inspection trip of the laboratory service of several VA hospitals. He greatly enjoyed making this tour, as he had every year since 1946, and was not to be deterred from going by the advice of anyone, even though suggestive changes appeared in electrocardiograms taken just before his departure. Known as "J. B." in his home town of Girard,



JAMES B. McNAUGHT
1894-1959

Kan., where he was buried on Aug. 11, and as "Jim" to his many and devoted friends, the latter often by his own direction to relatively young medical colleagues, he was the only person to have scored the "grand slam" of pathology by occupying the presidencies of the American Society of Clinical Pathologists, the American Board of Pathology, the International Academy of Pathology, and the American Association of Pathologists and Bacteriologists. He had the capacity to enjoy life to the fullest, which was hampered by a cerebrovascular accident in 1955. This

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left him with a peripheral field defect, which, fortunately, spared his central vision enough so that he could continue his keen use of the microscope. No pathologist enjoyed a wider friendship than he with those in his own field and also with many physicians in other branches of medicine and with laymen. The number of medical students, interns, and residents whom he helped, both with advice and in a financial way, is difficult to estimate, but they were many. He was a collector of fine paperweights and of medical stamps, on both of which he prepared two very beautifully illustrated talks, delivered at local and national meetings of pathology societies. His receipt of the seldom-bestowed Certificate of Merit from the American Society of Clinical Pathologists was a signal honor, indicating the high esteem of the pathologists of this country. For those wishing the many details of his full and varied career, the "Who's Who in America" may be consulted. His degree of M.S. in botany from the University of Kansas, his early teaching of bacteriology, and his experience as a medical technologist are noteworthy early accomplishments preceding his receipt of the M.D. degree from Stanford. For one who first knew him in 1934-1935 as an exchange instructor in pathology at the University of Rochester School of Medicine and Dentistry and was associated closely with him for 14 years, during his direction of the Department of Pathology at the University of Colorado School of Medicine, his passing has meant the loss of a kind and sincere friend. He was stricken while doing something which he greatly enjoyed and, fortunately, suffered little until about a half-hour before the end. In effect, he died with his boots on. The James B. McNaught Fund, for which many contributions have been received in the office of the Dean, is to be used toward the establishment of the James B. McNaught Library in the Department of Pathology at the University of Colorado School of Medicine.

RICHARD M. MULLIGAN, M.D.

News and Comment

ANNOUNCEMENTS

The University of Minnesota School of Dentistry announces the Third Annual Course in Advanced Oral Pathology for 1960. The course, held from Thursday, April 21, through Tuesday, April 26, 1960, devotes about half-time to examination of approximately 200 slides. Among the subjects and lecturers are the following:

1. Salivary Gland Pathology. Dr. John T. Godwin, Professor of Pathology, Emory University School of Medicine, Atlanta
2. Bone Pathology. Dr. Louis Lichtenstein, Chief of Pathology, Veterans Administration, Los Angeles
3. Advanced Roentgenographic Diagnosis of Diseases of the Jaws. Dr. Edward Stafne, Professor Emeritus, Mayo Clinic, Rochester, Minn.
4. Pathology of Soft Tissues. Dr. Richard Shuman, Chief, Department of Pathology, Norfolk General Hospital, Norfolk, Va.
5. Roentgenographic Techniques for the Head and Neck. Dr. Richard W. Moss, Chief, Oral Surgery Section, Veterans Administration Center, Wood, Wis.
6. Dermatopathology. Dr. Robert Goltz, Professor of Dermatology, University of Minnesota Medical School, Minneapolis
7. Odontogenic Tumors. Dr. Robert J. Gorlin, Professor, Oral Pathology, University of Minnesota School of Dentistry, Minneapolis
8. Oral Manifestations of Systemic Disease. Dr. Anand Chaudhry, Professor of Oral Pathology, University of Minnesota School of Dentistry, Minneapolis

The course will be limited to 20 participants. Tuition will be $100.00. Interested persons will please contact Dr. Robert J. Gorlin, Professor and Chairman, Division of Oral Pathology University of Minnesota School of Dentistry, Minneapolis 14.

PERSONAL

Dr. Granville Bennett's Appointment.—Dr. Granville A. Bennett, of the University of Illinois, has been appointed to the Veterans Administration Advisory Committee on Education.

Dr. Morgan Berthrong's Appointment.—Dr. Morgan Berthrong has been appointed Professor and Head of the Department of Pathology at the University of Colorado. He succeeds Dr. James B. McNaught, who continues in the department as professor of pathology.

Dr. Robert W. Wissler Awarded Honorary Degree.—Dr. Robert W. Wissler, Chairman of the Department of Pathology of the University of Chicago, was awarded the honorary degree of D.Sc., at the June, 1959, convocation of Earlham College.

Col. Frank M. Townsend Director of AFIP.—Col. Frank M. Townsend assumed the directorship of the Armed Forces Institute of Pathology on Aug. 1, 1959. He succeeds Capt. W. M. Silliphant. Captain Silliphant retired from the Navy on Sept. 1, 1959, and at that time joined the staff of the Cancer Research Institute, and became a member of the Department of Pathology of the University of California Medical Center in San Francisco.

Dr. Valy Menkin Resigns.—Dr. Valy Menkin has resigned as associate professor of experimental pathology at Temple University School of Medicine in Philadelphia. He will continue his work as a guest investigator at the Henry Phipps Institute of the University of Pennsylvania.

Dr. Paul D. Rosahn Visiting Professor of Pathology to Bangkok.—Dr. Paul D. Rosahn, associate clinical professor of pathology at Yale University School of Medicine, is serving as visiting professor of pathology at the University of Medical Sciences, Bangkok, Thailand, for one year, from Sept. 1, 1959.

DEATHS

Dr. Cornelius P. Rhoads.—Dr. Cornelius P. Rhoads, director of the Sloan-Kettering Institute for Cancer Research, New York, died on Aug. 13, 1959, at the age of 61. Dr. Rhoads was professor of pathology at the Cornell University Medical College until he became director of the Sloan-Kettering Institute.

SOCIETY NEWS

The American Society for Experimental Pathology.—The American Society for Experimental Pathology announces the following committee member lists for 1959-1960.

Meritorious Award or Honors Committee
- Dr. Thomas Kinney, Chairman
- Dr. Douglas H. Sprunt
- Dr. Sidney Madden
- Dr. D. Murray Angevine
- Dr. Charles E. Dunlap

Publications Committee
- Dr. Robert B. Jennings, Chairman
- Dr. J. F. A. McManus
- Dr. J. J. Lalich

Membership Survey Committee
- Dr. William H. Carnes, Chairman
- Dr. P. J. Fitzgerald
- Dr. H. I. Firminger
- Dr. J. G. Brunson
- Dr. J. R. Carter
- Dr. C. L. Yuile
- Dr. E. P. Benditt

Nutritional Pathology Committee
- Dr. Russell Holman, Chairman
- Dr. Richard Follis
- Dr. Conrad L. Pirani
- Dr. W. Stanley Hartroft
- Dr. Alvin J. Cox
- Dr. H. L. Ratcliffe

Representatives to Other Organizations

National Research Council, Division of Biology and Agriculture; Dr. Hans Schlumberger Division of Medical Sciences; Dr. R. L. Holman

American Association for Advancement of Science
- Dr. I. Davidsohn
- Dr. G. Haas

Eli Lilly Award Committee (Joint Committee with the Society of American Bacteriologists)

For Nominations: Dr. Alvin J. Cox (through 1959) Dr. Tom D. Hamilton (1960)

For Awards: Dr. Frank J. Dixon (through 1960)

Commission for Biological Stains
- Dr. J. F. A. McManus

Intersociety Committee on Increase in Research Pathology Potential
- Dr. Cyrus C. Erickson

Intersociety Committee on Public Information
- Dr. Robert W. Wissler

Representative to Federation Publicity Committee
- Dr. Robert B. Jennings

Representative of ASEP to Placement Committee
- Dr. Kenneth M. Brinkhous

Books

Normale und pathologische Entwicklung des menschlichen Herzens. By Priv.-Doz. Dr. Kl. Goerttler. Price, $7.85. Pp. 123, with 54 illustrations. Georg Thieme Verlag, Herdweg 63, (14a) Stuttgart N (American zone). (American agent—Grune & Stratton, Inc., 381 4th Ave., New York 16), 1958.

Causation and mechanisms of typical and atypical cardiac malformations are reexamined in this third fascicle of the series *"Zwanglose Abhandlungen aus dem Gebiet der normalen und pathologischen Anatomie."* The author's modified theory of the evolution of normal and malformed hearts is based on a detailed analysis of known data of cardiac embryogenesis, experiments with laminar flow through glass models of normal and malformed embryonal hearts, and serial sectioning of all stages of hearts of chicken embryos.

Goerttler stresses the following points: The decisive factor in the formation of the normal and the malformed heart is the shaping of the cardiac tube. Septum formation is a secondary phenomenon and is not the primary teleologic purpose of the cardiac development. He introduces the concept of "enteric" circulation, which is the blood flow from the arteries arising ventrally from the descending aorta and their corresponding veins. In his opinion the separation of the systemic and pulmonary circulation is preceded by a qualitative separation of "somatic" and "enteric" circulation in primitive vertebrates. The quantitative separation is achieved by limiting the original "enteric" circulation to the lungs alone, and not by mere crossing of the aorta and pulmonary artery. Cause of cardiac malformation is solely the arrest of growth at different stages of development, caused by exogenous and endogenous factors. Areas with most active growth (mitosis) are damaged most readily. Deviation from the main course of ontogenesis as found in phylogenesis is the form of specialization (for instance, the two aortas in the crocodile) plays no role as a cause of malformed hearts. A number of autopsy findings illustrate typical instances of the various cardiac malformations.

Goerttler's theory differs substantially from previous theories and is based almost exclusively on hemodynamic factors. Many of the explanations of the ontogenesis of different malformations are very convincing. The book is written in clear German. The cited literature is mainly German and Anglo-American but includes some French. The book can be recommended.

Carcinogenesis by Ultraviolet Light. By Harold F. Blum. Price, $6.50. Pp. 340, with illustrations. Princeton University Press, Princeton, N.J., 1959.

The author of this book is a member of the National Cancer Institute and at present visiting professor of biology at Princeton University. His present work gives much more than the title indicates. First, it presents a comprehensive survey on biological effects of ultraviolet rays in general. Second, as an introduction to the main topic of carcinogenesis, it offers a lucid discussion of replication and cell division. Finally, in discussing carcinogenesis the author does not restrict himself to the subject of ultraviolet radiation-induced cancers, but from his experimental data and calculations he develops what he hopes may be a generally valid unifying theory of carcinogenesis or, at least, a theory for all cancers due to environmental causes.

The book is written with an uncanny literary talent, most unusual among scientists, and in such a delightful, easy style that it is not only an intellectual but also an esthetic pleasure to read it. Most chapters are introduced by wittily chosen, pertinent mottoes taken from writers and philosophers from all over the world, old and modern. But, in spite of its light-handed, smooth style, the book is written with absolute precision and follows a perfectly logical train of thoughts. Facts, conclusions from facts, and hypotheses are strictly distinguished as such.

A great virtue of the book is the way it handles mathematical operations. While the book can rightly be called "a study in quantitative biology"—and Dr. Blum is well known to be a master of "biological mathematics"—those many, less fortunate biologists who cannot follow all the intricacies of calculus do not lose out, for the text renders the points which are deducted mathematically perfectly understandable.

The book has a "personal slant," as the author expresses it; but it could not be otherwise, because almost the whole volume deals with the author's own work. Since the publication

of his first monograph, "Photodynamic Action and Disease Caused by Light," in 1941, Dr. Blum has been a nationally and internationally acknowledged leading expert and prolific worker in all fields pertaining to the biological effects of ultraviolet light. His newest book is a peak achievement in his distinguished career.

In the general discussion of ultraviolet effects, one chapter deals with the postulate that, in order to pinpoint the "chromophore," i.e., the compound in a tissue the photochemical reaction of which elicits the biological effect, the absorption spectrum of the incriminated compound and the action spectrum of the biological effect must be identical. In a fine analysis it is shown that this postulate can be fiulfilled only in simple situations. Still, it is demonstrated that some information can be obtained on this basis if one tries to differentiate primary effects on proteins and on nucleic acids.

Other chapters deal with the following topics: absorption and penetration of various ultraviolet wavelengths in different tissues; dose-rate dependence; energetics of photochemical reactions; inactivation and lethal action on enzymes, viruses, and micro-organisms; effects on cell membranes resulting in cytolysis ("lysogeny"); photodynamic action requiring the presence of oxygen; comparison of ultraviolet effects with those of higher energy-ionizing radiations; photorecovery by irradiation with rays of longer wavelengths. The great differences in human and mouse skin are duly emphasized and described. The sunburn and sun tan reactions in man, their action spectra, the mechanism of immunity to sunburn, and photorecovery are subjects of novel analyses.

Concerning cell division, energetics, and morphology are discussed with equal clarity. Description of the delaying action of ultraviolet rays on cell division is based on the author's classical experiments. He also discusses the locus of action and the nature of the refractory period. There are excellent digressions on the concepts of mutation, chromosome aberrations, polyploidy, cross-over, somatic mutations, adaptation, and differentiation. As a background to all these more general chapters, the author ingeniously utilizes up-to-date results of biophysics, biochemistry, and biogenetics.

In the chapters dealing with ultraviolet-induced cancers, the experiments are first described in detail. The results are remarkably well reproducible. They clearly show that within wide limits the same total energy will cause cancerization independently of the dosage schedule (so-called reciprocity of dose-rate dependence). The results are evaluated in such a way that a general theory of environmental carcinogenesis emerges. "Cancer is the expression of a tendency for certain clones of tissue cells to proliferate faster than their fellows, this tendency being inherited within the clone." Cancerization by ultraviolet light is a continuous, uninterrupted process with a constant acceleration of growth rate. This acceleration indicates addition of new cell clones also undergoing accelerated division. Extrapolation of the numerical experimental data shows that the initial volume from which the tumor arises is much smaller than a cancer cell. The author calls the basic replicated unit of subcellular dimension a "tem," on the assumption that these units are templates. Because ultraviolet radiation damages cells and increases their permeability, one may assume that the cells which are least injured profit at the expense of their more severely damaged neighbors, the survivor cells increasing their supply of tems from the moribund, decomposing cells; hence the accelerated rate of growth. It is well possible that the tems are nucleic-acid templates. There are good reasons, however, to believe that they are not virus particles, mainly because the tems are copied in controlled amounts and accelerate their rate of replication together with that of their host cells, while viruses seem to run away from the control of the cell and kill it. The experimental results and the calculations made therefrom do not support the view that the tem is a mutated gene, partly because carcinogenesis is a continuous cumulative event, not a saltatory one with a sudden onset. Also, it does not occur stepwise or in stages, as Berenblum's concept would postulate. Neither do the experiments support the view that cancer arises because of the breakdown of some mechanism which regulates cell division.

Indeed, all data indicate that cancerization is a quantitative rather than a qualitative change, consisting in a faster rate of proliferation on the part of certain clones of tissue cells, this faster rate being inherited in subsequent generations. In recent experiments Paschkis, Bielka, Latarjet, and their associates have shown that massive injection of nucleic acids from the same species of animals may produce cancers. These observations provide supporting evidence for the author's concept.

It is indeed hard to find faults in this magnificent book, a monumental contribution to modern cancer research. But if there should be any criticism, it may be mentioned that, in

contrast to all the rest of the book, one or two clinical implications are handled in a not entirely precise manner. Both cutaneous squamous-cell and basal-cell carcinomas are said to be relatively little malignant, less invasive, and to metastasize less often than other cancers. The truth is that basal-cell carcinomas, though often extremely invasive, practically never metastasize, while squamous-cell carcinomas of the skin may be as malignant as any other type of cancer. The reviewer is at variance also with the view that the concept of "precanceroses" is misleading. The concept not only is based on well-established clinical facts but also is compatible with the author's findings and conclusions.

In way of criticism, it also may be pointed out that there are more than the usual number of misprints, some annoying, some funny, such as "respectfully" instead of "respectively," on page 290. We find "activation" instead of "inactivation" on page 25; in Figure 9, on page 43, left and right are interchanged; on page 59 the word "keratosis" is used, when obviously "keratinization" is meant; on page 63 the name of Raab is misspelled; on page 138 "psychological" appears instead of "physiological," and on page 139 "homopoetic," instead of "hemopoetic." It may seem petty to enumerate all these tiny imperfections in a great work, but they are listed in the hope that they will be corrected in subsequent printings.

Dr. Blum's book is a "must" for all experimental biologists, pathologists, and others in medical research.

Addendum: Since completion of the review misprints have been corrected in an "ERRATA slip" by the publisher.

Phenacetinabusus und Nierenschädigung. By Prof. Dr. H. Sarre; Doz.-Dr. A. Moench, and Dr. R. Kluthe. Price, $3.50. Pp. 109, with 28 illustrations. Intercontinental Medical Book Corporation. Georg Thieme Verlag, Herdweg 63, (14a) Stuttgart N (American Zone). (American agent—Grune & Stratton, Inc., 381 4th Ave., New York 16), 1958.

Between 1950 and 1953 Swiss authors, notably Spuehler and Zollinger, called attention to a special type of nonobstructive nephritis, the so-called chronic interstitial nephritis. Clinically, this entity is characterized by anemia, azotemia, normal diastolic blood pressure, little albuminuria, and leukocyturia. In the original series of Spuehler and Zollinger, a conspicuous relationship between the abuse of habit-forming analgesics, particularly, acetophenetidin U.S.P. (Phenacetin), and the appearance of nephritis is suggested. Since then many more cases of chronic interstitial nephritis, presumably due to Phenacetin abuse have been published in Switzerland and Germany.

A symposium on "Phenacetin Abuse and Renal Damage" was held in Freiburg, Germany, in January, 1958. Under the editorship of Prof. H. Sarre, the proceedings of this colloqium are published. Three main topics comprise the pathological anatomy, pharmacology, and clinical use of acetophenetidin and chronic interstitial nephritis. The main referents are Uehlinger, Zurich; Kuschinsky, Mainz, and Moeschlin, Solothurn.

It is not possible to summarize all the papers in a brief review. As usual, much is gained by reading the discussions of active workers in renal physiology and pathology in Mid-Europe. Certain conclusions can be drawn. Abuse of certain analgesics taken in high doses over many years, and perhaps decades, causes renal damage. The nature of the produced lesion is uncertain, and much controversy exists about the causal role of acetophenetidin.

The controversies as to whether acetophenetidin produces chronic nephritis and whether or not patients with renal disease take large doses of analgesics, which, in turn, may be an additional factor in renal injury, could not be resolved.

The Management of Oral Disease. Edition 2. By Joseph L. Bernier, D.D.S., M.S., F.D.S., R.C.S. Price, $15. Pp. 875, with 1,031 illustrations and 5 color plates. C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1959.

This volume is the second edition of a textbook of oral pathology by the well-known oral pathologist who is the Chief of the Oral Pathology Division, Armed Forces Institute of Pathology.

The material is organized under twenty-two chapters, each chapter dealing with the pathology of a particular structure or with pathologic processes that have similar oral manifestations. The material is presented in a concise and understandable manner. The author repeatedly emphasizes the relationship of the systemic or general condition to the local or oral condition. Although specific diseases are dealt with individually, the author attempts to give a conceptual approach to pathological processes in the introduction of each chapter.

The chapter on salivary glands has been extensively revised, and new concepts on the histogenesis of lymphoepithelial lesions are presented. The author has also presented concepts on the pathogenesis of leukoplakia and cementifying fibroma that are not generally accepted at the present time. Some rather convincing evidence is given to support these concepts. With the increased use of high-speed instrumentation in dental practice, the inclusion of a section on the pulpal responses to this technique is particularly well timed.

The classification of cysts of the oral regions, odontogenic tumors, and salivary-gland tumors has been modified by the author, adding clarity to the classifications.

The author's style is straightforward and readable. There is an abundance of well-done gross, roentgenographic, and microscopic illustrations. There is a good bibliography at the end of each chapter. The type is easy to read, and the book is well bound, with a durable and attractive cover. The book is recommended for undergraduate students, both dental and medical, and for general practioners of dentistry.

Krebsforschung und Krebsbekämpfung. Volume 2. By Prof. Dr. H. Martius, Göttingen, and Doz.-Dr. H. Hartl, Göttingen. Price, D.M. 45. Pp. 364, with 137 illustrations. Urban & Schwarzenberg, Thierschstrasse 11, München 26; Meinekestr. 13, Berlin W. 15, 1957.

This book represents the proceedings of the fifth meeting of the German Central Committee for Cancer Research and Cancer Therapy, held in April, 1957. Forty papers are abstracted in this second volume. The majority are reviews on general aspects of cancer; clinical studies and only a few original experimental papers are included.

The first day of this meeting was dedicated to summational presentations, such as carcinogenesis (Muehlbock, Amsterdam), carcinogenesis as a biological problem (Bun-Hoi, Paris), metabolism and endogenous carcinogenesis (Dannenberg, Munich), and constitution and cancer (Dormanns, Solingen). Biochemical studies on tumors follow: cytochromes in Ehrlich ascites tumor cells (Hess, Heidelberg) and mitochondria in tumor cells (Weissenfels, Bonn). Three presentations from Professor Graffi's group on the oncogenic activity of cell-free filtrates are of interest to those concerned with the virus etiology of cancer. Papers on carcinogenesis by physical means (Huenkel, Hamburg), the light-sensitizing O_2 transmission as common action of phototoxic dyes and carcinogenic hydrocarbons (Schenk, Göttingen) and carcinogenic substances in tobacco smoke (Neukomm and Bonnet, Lausanne) conclude the first day.

Professor Schinz' (Zurich) thorough account of modern methods of cancer therapy opens the second day. Some 25 papers follow, mostly dealing with treatment and prognosis of uterine cancer and the chemotherapy of other tumors. In general, the volume is valuable as a record of progress.

Atlas of Tumor Pathology, Sec. 1, Fasc. 2: Tumors of the Skin. By Herbert Z. Lund, M.D. Price, $3. Pp. 330, with 265 illustrations. Armed Forces Institute of Pathology, Washington 25, D.C., 1957.

This is a comprehensive account of skin tumors. It should be of special interest to any practicing pathologist and dermatologist. In the excellent introduction, Dr. Lund covers such controversial fields as nomenclature, etiology of skin tumors, and histogenesis of epithelial tumors of the skin. Fortunately, many tumor-like conditions are included for the sake of differential diagnosis. The text is restricted to the most essential features, since almost all lesions are amplified by supreme black-and-white and color illustrations. Many pathologists will find this fascicle of great value.

Atlas of Tumor Pathology, Sec. V, Fasc. 20: Tumors of the Esophagus. By Arthur Purdy Stout, M.D., and Raffaele Lattes, M.D. Price, $1. Pp. 105, with 58 illustrations. Armed Forces Institute of Pathology, Washington 25, D.C., 1957.

Of all esophageal tumors, the commonest and most important, carcinoma, is usually easy to interpret. Therefore, in this fascicle, Drs. Stout and Lattes describe at some length the rare benign tumors, cysts, and malformations. Squamous-cell carcinoma is extensively described and demonstrated. A final chapter on sarcoma, carcinosarcoma, and malignant melanoma is included. The many black-and-white and color photomacro- and photomicrographs are excellent. This fascicle is of a high standard similar to that of the previous ones edited by Dr. Stout.

Die Gefässarchitektur der Niere. By Prof. Dr. A. von Kügelgen, Dr. B. Kuhlo, Dr. W. Kuhlo, and Dr. Kl.-J. Otto. Price, $11.20. Pp. 112, with 89 illustrations. Georg Thieme Verlag, Herdweg 63, (14a) Stuttgart N (American Zone). (American agent—Grune & Stratton, Inc., 381 4th Ave., New York 16.) 1959.

Volume 5 of this series deals with the architecture of the dog kidney. The booklet is divided into three parts: the arterial, the venous system of the dog kidney, and the interrelations of the arterial and venous systems. The results of the applied experimental techniques (injection of plastic material and India ink) can be summarized as follows: All arteries are end-arteries; all veins (with the exception of the cortical veins) are continuous. Venous valves are found toward the corticomedullary junction. A separate course of veins and arteries is found in the cortex, while the medullary vasculature has a common course. In the most superficial cortex only venous channels are present.

The authors are to be congratulated on a meticulously performed study.